

THE ACTION OF STRUCTURAL ANALOGUES

OF NICOTINE

on

SYNAPTIC TRANSMISSION

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by

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The Relevance of Nicotine to the Study of  
Synaptic Transmission

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The Relevance of Nicotine to the Study of  
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I

Action of Nicotine on Transmission in Autonomic  
Ganglia:-

In 1863 Traube (see Heubner 1947) showed that nicotine slowed the dog's heart even after the vagus nerve had been cut and further that, after this action of nicotine, electrical stimulation of the vagus did not slow the heart.

Schmiedeberg (1870) showed that atropine prevented the initial slowing effect of nicotine, and that, if atropine had not been given, muscarine would still slow the heart during the phase when electrical stimulation of the vagus was ineffective. Schmiedeberg and Traube concluded that nicotine stimulated and then blocked some connecting link between the vagus trunk and the site of action of atropine.

In /

In 1889 Langley and Dickinson showed that the site of action of nicotine was the autonomic ganglia. They applied a 1% solution of nicotine to the superior cervical ganglia of the cat and produced pupillary dilatation, the same response obtained by electrical stimulation of the cervical sympathetic chain. Nicotine applied to the nerve trunks on either side of the ganglia did not produce this effect. After the stimulatory effects of nicotine had passed he noted that electrical stimulation of the sympathetic nerve trunk no longer produced pupillary dilatation although electrical stimulation of the postganglionic trunk did.

Langley concluded from these observations that nicotine stimulated and then blocked the ganglionic elements in the sympathetic outflow.

Using nicotine as a pharmacological tool Langley 1889 et seq. mapped out the sites of autonomic ganglia in the cat. If nicotine caused a stimulation followed by a block at a ganglion this was taken as evidence of the presence of a synapse. If nicotine was without effect it was concluded that the fibres /

fibres did not synapse at this point. The action of nicotine at ganglia, therefore, is extremely important because it was this action which was used to reveal the existence of ganglia.

Langley (1901) showed that nicotine still stimulated the sympathetic ganglia after the preganglionic nerve had been cut and allowed to degenerate. He deduced from this that the site of the paralysing action of nicotine on the ganglia was the cell surface and not the nerve endings.

The observation that such drugs as nicotine and esters of choline could mimic the effects of nerve stimulation eventually led to the consideration of the possibility that transmission in the ganglion might proceed through a physiological chemical transmitting substance. This idea had already been proposed for transmission from the parasympathetic postganglionic nerve fibre. (Hunt and Taveau 1906, Dixon 1907).

Dale (1914) showed that there were differences between the action of drugs on the ganglia and the postganglionic parasympathetic site. The alkaloid muscarine was active on the postganglionic parasympathetic site and inactive at /

at the ganglia whereas the reverse was true for nicotine. Acetylcholine which acts at both of these sites was in fact shown to be the transmitter at the end of the postganglionic parasympathetic nerve fibres. (Loewi 1921, Dale and Dudley 1929, Dale and Gaddum 1930, Dale 1933).

Kibjakow (1933) described experiments in which he had artificially perfused the superior cervical ganglion of the cat and found that, when the preganglionic nerve was stimulated something appeared in the venous fluid which acted as a stimulus to the ganglion cells on reinjection, as shown by contraction of the nictitating membrane. This was the first real evidence that a chemical substance was responsible for transmission across the synapse of a ganglion. Chang and Gaddum (1933) from their own observations of the presence of an acetylcholine-like substance in extracts of sympathetic ganglia suggested that this substance might be acetylcholine.

Feldberg and Minz 1933 showed that when the splanchnic nerve supply to the adrenal medulla was stimulated acetylcholine could be detected in /

in the venous blood if eserine was present to prevent its destruction. Feldberg and Gaddum (1933) using Kibjakow's technique added eserine to the Locke's solution perfusing the ganglia and showed beyond doubt that the active substance appearing in the venous fluid during preganglionic nerve stimulation was acetylcholine.

The transmitter in autonomic ganglia therefore has been shown to be acetylcholine whose actions at this site are imitated by nicotine (Dale 1914). Feldberg and Vartiainen (1935) showed that there was a considerable similarity in the stimulant activity of these two compounds on the ganglia. They used the perfused superior cervical ganglion of the cat as described by Feldberg and Gaddum (1934) and added nicotine to the perfusion fluid. They found that the threshold for the stimulant action of nicotine was 100 ng. and for acetylcholine was 1 to 3  $\mu$ g. in the absence of eserine. The threshold dose for acetylcholine when it was protected by  $10^{-6}$  w/v eserine was 50 to 200 ng. which is quantitatively similar to the threshold for nicotine.

Feldberg and Vartiainen (1935) further showed, /



showed, in agreement with Langley(1901), that the site of the blocking action of nicotine was at the cell surface and not the nerve endings for they showed that the release of acetylcholine from the nerve endings was unimpaired during nicotine block.

The blocking action of nicotine on the ganglia has been further studied by relatively few workers. Koppanyi, Dille, and Linegar (1936) gave physostigmine (0.2-0.35 mg./Kg. i.v. femoral) to animals which had been given sufficient nicotine (2-10 mg. nicotine salicylate/Kg. i.v.) to block the superior cervical ganglion, and claimed that this abolished the nicotine block, as shown by the return of the normal response to preganglionic nerve stimulation (i.e. dilatation of the pupil, exophthalmous withdrawal of the nictitating membrane, widening of the palpebral fissure and occasional rises in blood pressure). They supposed that nicotine (and curare) caused a ganglion block by interfering with the production of acetylcholine by the nerve endings. The evidence, however, is that nicotine does not prevent the release of acetylcholine (Langley 1901, Feldberg and Vartiainen 1935).

The /

The reversal of nicotine block by eserine could be explained if nicotine were acting as a competitive blocking agent, but there is no evidence (see below) that this is so. In these circumstances the reversal of the block of nicotine by eserine requires further study particularly as the results of Koppanyi et al were based on experiments on only a small number of animals, (3 dogs, 4 cats, and 3 rabbits) and their experimental technique makes it difficult to tell how much eserine reached the ganglia. It is also difficult to tell whether the eserine was given during a complete block or during an early recovery phase from nicotine block, a factor which may be important in deciding whether this second phase of block is truly competitive or is due to a desensitisation of the "receptors" to transmitter.

A physiological study of the mode of nicotine block at the ganglia was made by Paton and Perry in 1953. They stimulated the cut preganglionic sympathetic cervical nerve of a cat at varying frequencies and recorded the action potentials produced in the postganglionic nerve by means of external nonpolarisable electrodes (one lead looped round the body of the ganglia and the other placed at the point where /

where the postganglionic<sup>8</sup> nerve trunk had been tied and cut). Drugs were given in 0.2 ml. retrogradely intra-arterially or intra-venously.

They found that large doses of nicotine, acetylcholine, and tetramethyl ammonium all produced a depolarisation of the ganglion accompanied with a reduction of the spike height of the action potentials. They noted, however, that the depolarisation caused by nicotine was much more transient than that caused by the other two drugs and that the spike potential, in one experiment with 300  $\mu$ g. nicotine i.a. did not reappear for 10 minutes, nor return to normal for 30 minutes after depolarisation had passed off. In a similar experiment with tetramethyl ammonium the recovery from depolarisation was much slower and almost paralleled the recovery of the spike height to normal.

These observations suggested to them that nicotine may have a mixed action: partly acetylcholine-like and partly by antagonising the effects of acetylcholine.

To test this hypothesis they gave a dose of 200  $\mu$ g. of nicotine i.a. and repeated this 4 minutes later when the depolarisation caused by the first dose had almost passed off and they found that only an eighth of the original depolarisation was produced. A third dose of 200  $\mu$ g. nicotine given 9 minutes later when the spike potential had returned to 50% of normal produced /

produced 50% of the original depolarisation. The presence of a block without depolarisation in which there is resistance to further depolarisation by nicotine suggested to them a competitive type of block.

That this is a form of competition in the sense that the continued presence of the first dose at the sites of action ("receptors") prevents the second dose from producing its action (depolarisation) can neither be readily accepted nor disproved from the evidence given. A test showing that this was a truly reversible form of competition (cf. atropine and acetylcholine at muscarinic site) would have been to show that there was a quantitative increase in the extent of the depolarisation produced (at the same stage of the second phase of nicotine block) by successively doubling the second dose of nicotine given. Without such a test being performed one may equally well conclude from the evidence given that the second phase of block by nicotine was due to a "desensitisation" of the receptors of the ganglion surface to depolarising drugs (cf. the evidence given below for the neuro-muscular junction). During this phase the receptors would be refractory to further /

further doses of nicotine and it would be only on recovery to their "sensitive" state that further depolarisation would occur. In such a block it can be seen that the percentage of "sensitive" receptors at any given stage in recovery would be the factor which determined the degree of depolarisation produced by further doses of nicotine. If this was the case only one degree of depolarisation could be produced no matter how large a dose of nicotine was applied at any one stage during recovery.

From the results given by Paton and Perry it can be seen that maximum depression of the spike potential (and presumably ganglion blockade) occurred within the time required for the depolarisation produced to reach a maximum. This is of importance in this work as it is the initial degree of block (during depolarisation) which is taken as an index of nicotine-blocking activity and not the duration of block which would presumably be more dependent upon the extent of phase II block. Large doses of acetylcholine (1 mg. i.v.) and tetramethyl ammonium (300 $\mu$ g. i.a.) showed a similar relationship in time between the onset of maximal depolarisation and depression of spike potential.

The /



The relationship, if any, between the depolarisation produced and the degree of block present is not a simple one in the case of nicotine. This is also true, however, for the block caused by large doses of acetylcholine. Paton and Perry studied the time courses of the development of, and the recovery from, both depolarisation and block. They found that the reduction of the spike height was greater for a given degree of depolarisation the longer the depolarisation had been present.

Thus, although acetylcholine differs from nicotine in that depolarisation appears to remain for the duration of block there is an appreciable return to the nondepolarised state whilst the block continues with a much slower rate of recovery, (e.g. from Figure 4 the depolarisation expressed as a percentage of the initial spike height recovered from about 170% to 50% whereas the block expressed as the percentage reduction of the spike height had only recovered from 75% to 60% in a similar time). From these observations acetylcholine appears to hold an intermediate position between nicotine and tetramethyl ammonium. There may thus be a greater resemblance between the blocking action of /



of nicotine and acetylcholine than is at first sight apparent.

Eccles (1956) studied the action of nicotine on the isolated superior cervical ganglion of the rabbit. She used low concentrations which produced a sustained depolarisation of the ganglion and studied the change this produced in the spike and the after potentials to preganglionic nerve stimulation. The characteristic effect was a depression of the negative (N) wave and an increase in the after positivity (P wave). The meaning of these effects is still obscure although it has been suggested that the P wave may be due to a prolonged high potassium conductance of the cell membrane (cf. Hodgkin and Huxley 1952). This P wave also follows after the motoneurons have been depolarised by extrinsic currents, i.e. externally applied currents from electrodes on the cell surface (Coombs, Eccles and Fatt, 1955). Larger doses of nicotine were found to suppress both the spike and after potentials.

These results on the rabbit ganglion are in agreement with those obtained on the application of nicotine to the cat's superior cervical ganglion /

ganglion, (Eccles 1955 a and b), but as stated above the meaning of the externally recorded after potentials is still unknown and thus no valuable conclusions on the action of nicotine may be drawn from this work. Evidence is required on the action of nicotine on the potentials recorded with intracellular electrodes.

To conclude, the study of the action of nicotine on the ganglion has been of great importance historically, and the resemblance of its effects to those of the physiological transmitter acetylcholine leave little doubt that an understanding of the action of the one will be useful in the understanding of the other.

## II

The Action of Nicotine on Transmission at the Neuromuscular Junction:-

At the neuromuscular junction nicotine has a twofold action. There is an initial stimulation which may be either contraction or contracture (see below) followed by a block of transmission.

Chemical Transmission at Neuromuscular Junction.

The chemical transmitter at the neuromuscular junction, like that at the ganglion, has been shown to be acetylcholine. Hess (1923) perfused the voluntary muscles of frogs and noted that, when he stimulated the nerve supply, an acetylcholine-like substance appeared in the perfusate. Dale (1934) removed the superior cervical ganglia of a cat and allowed the autonomic nerves to the tongue to degenerate. He perfused the tongue with Locke's solution containing eserine and found that when the motor nerves were stimulated acetylcholine appeared in the perfusate. Dale, Feldberg and Vogt (1936) cut the motor nerves supplying various skeletal muscles of the cat and found that denervated muscles did not release acetylcholine into the perfusion fluid when the muscle was stimulated directly, whereas innervated muscle did. This showed /

showed that the acetylcholine found in the perfusate after nerve stimulation was released from the nerve endings and gave added support to the now accepted theory that transmission across the synapse at the neuromuscular junction is mediated by the chemical transmitter, acetylcholine.

#### Stimulant Action of Nicotine.

- (a) Contracture. The effects of acetylcholine and nicotine on striated (voluntary) muscle are complex and extremely variable. On certain muscles of the frog, tortoise, and bird, acetylcholine and nicotine produce a slow long-lasting contraction of low tension: this has been called a contracture to distinguish it from the rapid twitch contraction normally produced in voluntary muscle by motor nerve stimulation. Riesser and Neuschlosz (1921) showed that contracture was produced by acetylcholine in low concentrations ( $10^{-5}$  w/v for frog rectus). Sommerkamp (1928) and Wachholder and Ledebur /

Ledebur (1930) showed that in the frog and tortoise the muscles which produced contracture in response to the application of acetylcholine were those considered to be most important for the maintenance of normal posture.

- (b) Contraction. Contracture is not the only form of stimulant response shown by skeletal muscle in response to nicotine-like drugs. Nicotine and acetylcholine when injected rapidly into certain voluntary muscles produce an initial quick contraction. For instance Langley (1907) showed that on the frog sartorius nicotine ( $10^{-2}$  to  $10^{-5}$  w/v) produced twitches and a quick type of contraction, resembling a short tetanus, prior to the slowly developing contracture. Feldberg and Minz (1931) and Brown Dale and Feldberg (1936) also observed quick contractions of normal mammalian muscles when moderate doses of acetylcholine were injected close arterially. /

arterially. (20 $\mu$ g. into the gastrocnemius muscle of a spinal cat).

Presence of Two Types of Fibres.

- (1) Frog. These results suggest that there are two types of muscle fibres: one which responds with a rapid twitch, and the other which develops a slow contracture on the application of nicotine-like drugs. Katz and Kuffler (1941) and Kuffler and Vaughan Williams (1953 b) obtained evidence that this was so: they demonstrated the presence, in frog muscle, of "slow" muscle fibres which differ from the "fast" twitch fibres in that they have a lower membrane potential, and they never give propagated muscle action potentials on nerve stimulation.
- (2) Chicks. Chicks have been used for some time (Buttle and Zaimis 1949, Child and Zaimis 1954) to distinguish between two types of neuromuscular blocking agents. One type, of which decamethonium is typical (see below) produces a contracture of certain muscles, especially those in the neck. Isolated /



Isolated preparations of the semispinalis (Child and Zaimis 1954) and the biventer cervicis (Ginsborg and Warriner 1960) have been used for qualitative and even quantitative investigations of neuromuscular blocking agents. Ginsborg (1959 and 1960) has shown that the biventer cervicis contains two types of fibre, one of which responds to acetylcholine and to depolarising drugs like decamethonium by producing a contracture whereas the other gives a contraction in response to nerve stimulation and is blocked by neuromuscular blocking agents of either type.

Ginsborg (1960) has deduced from a study of the spontaneous activity recorded from muscle fibres in the chick that the type which produces contracture is supplied by a number of axons and has neuromuscular junctions distributed at many points along it, whereas the twitch fibres are supplied /

supplied by a single axon with a focal end-plate. The "slow" fibres of the amphibian muscles have also been shown to have a diffuse form of innervation (Kuffler and Vaughan Williams 1953a, Burke and Ginsborg 1956, Burke 1957). One difference between the "slow" amphibian muscle fibres and the corresponding "contracture" fibres in chick is that the latter can give a propagated action potential on nerve stimulation (Ginsborg 1959).

#### Connection between Depolarisation and Contracture.

Ginsborg (1960) has pointed out that the distance between adjacent junctions in the diffusely innervated fibres of the chick is, at the most, of the same order of magnitude as the space constant of the fibre. Thus, pharmacological agents which cause depolarisation at the neuromuscular junction may be expected to produce a relatively uniform depolarisation of the surface of such a muscle fibre. Ginsborg concludes that if contracture is intimately related to depolarisation it seems likely that it is these fibres which are responsible for the /

the contracture of such avian muscles.

In their physiological behaviour these slow fibres are clearly quite different from those which produce a twitch response.

#### Pharmacological difference between Fibres

A pharmacological difference between the two types of muscle fibre is revealed by the action of antagonists. Raventos (1937) found that atropine abolished the contracture but had no effect on the twitch whereas curarine (a mixture of the alkaloids from tube curare) decreased or abolished the twitch but had no effect on contracture.

#### Quantitative Comparison of the Neuromuscular Junction and the Ganglion:-

The stimulant activity of nicotine on normal voluntary muscle is a hundredth of that of acetylcholine, for Bacq and Brown (1937) showed that an injection of 500 $\mu$ g. into the artery supplying the gastrocnemius or the tibialis anticus of the cat caused a rapid contraction equivalent to that caused by 5 $\mu$ g. acetylcholine. This differs from the situation at the ganglion where nicotine is approximately as effective as acetylcholine as a stimulant of ganglion cells. (Feldberg and Vartiainen 1934).

## BLOCKING ACTIVITY

### Introduction

In higher concentrations the stimulant action of nicotine is normally followed by a block of transmission. Langley (1907) showed that nicotine causes a block at the neuromuscular junction in doses which left both the nerve and muscle fibres functionally responsive. This implies that the drug blocks by an action at the end-plate region. All drugs like nicotine which cause contraction of striated muscle also have this paralysing action if they are allowed to remain in contact with the muscle fibres. Bacq and Brown (1937) observed that on the cat gastrocnemius and tibialis muscles the paralysis produced by nicotine could be reversed completely by a tetanus or by the application of the same concentration of acetylcholine which normally caused a twitch.

### The effects of Decamethonium on the end-plate

The nature of the block caused by drugs at the neuromuscular junction has been studied to a greater extent than on the ganglion, and it has been shown that not all drugs cause an initial stimulation before block ensues. (Paton and Zaimis 1949, 1950 and 1952). Burns and Paton (1951) /

(1951) showed that the block caused by (+)-tubocurarine differed from that caused by decamethonium in that (+)-tubocurarine did not cause a depolarisation of the end-plate region of the gracilis muscle fibres of the cat whereas decamethonium caused a longlasting depolarisation. Burns and Paton observed the following effects of decamethonium on the gracilis muscle: (i) it produced a depolarisation of similar duration as the block (Paton and Zaimis 1949); (ii) the end-plate during block did not respond to direct electrical stimulation; (iii) the conduction of a muscle action potential along the fibre was stopped at the end-plate region during block; (iv) the propagation threshold of the end-plate was raised; (v) artificial repolarisation of the end-plate region reversed the block.

The effect of (+) Tubocurarine on the end-plate

Earlier work by Eccles, Katz, and Kuffler (1941) and others showed that the block caused by "curare" at the neuromuscular junction was due to an increase in the threshold of the end-plate to acetylcholine with no concomitant depolarisation. Burns and Paton (1951) showed that in the gracilis muscle of the cat this block /

block differed from that caused by decamethonium in that conduction of the muscle action potentials was not stopped at the end-plate region and that the threshold for the propagation of the spike action potential from the end-plate potential was only  $\frac{1}{3.5}$  times the threshold for decamethonium.

#### Classification into two types of block

Burns and Paton concluded that whereas (+) - tubocurarine blocked by increasing the threshold of the end-plate to acetylcholine, decamethonium blocked by producing a persistent depolarisation of the end-plate region.

In support of the idea it was noted that the block caused by one of these types of drugs could be reversed by the application of one of the other type. For instance Hunter and Pascoe (1951) found that moderate doses of decamethonium antagonised the effects of (+) - tubocurarine on the tibialis anterior and the gracilis muscles of the cat. Larger doses caused an increased block. Burns and Paton (1951) showed that (+) - tubocurarine applied to a muscle which was blocked with decamethonium, restored the propagation of an action potential. Neuromuscular block, however, /



however, returned when the end-plate potential had been reduced by a third.

An attractive feature about this simple classification of drugs into "curare-like" and "decamethonium-like" is that the action of eserine on the two types of block can be explained. For instance, Paton and Zaimis 1949 noted that on the cat tibialis eserine reversed the block caused by (+) - tubocurarine (presumably because the increased concentration of acetylcholine competitively displaced the (+) - tubocurarine from the sites of action, "receptors") whereas eserine did not reverse but sometimes even increased the block due to decamethonium (presumably because the increased concentration of acetylcholine caused further depolarisation and thus increased the block).

Further support for the hypothesis was the observation that the sensitivity of different muscles to decamethonium not only varied from species to species (Paton and Zaimis 1952) but depended upon the type of muscle. There appeared to be a reciprocal relationship between /

between the sensitivity of a given muscle to the two types of blocking drug. For instance Paton and Zaimis 1951, and Zaimis (1953) showed that the stimulant action of decamethonium on the normal soleus (a "red" muscle) was more persistent than on the normal tibialis (a "white" muscle) whereas (+) - tubocurarine was more effective in blocking "white" muscle than it was "red" muscle.

The two Phases of block produced by Decamethonium.

The action of decamethonium, however, cannot be regarded in all species as one of pure depolarisation. Jenden, Kamijo, and Taylor (1951) observed that decamethonium had a "biphasic" blocking action on the isolated rabbit lumbrical muscle. The first phase of block had the properties of decamethonium in vivo and the second phase with a slower rate of onset progressed to a steady level. They found that this second phase of block could be reversed by increasing the potassium concentration, and by adding eserine. Zaimis (1953) studied the action of decamethonium and succinylcholine on eight different species and /

and found that on certain muscles (e.g. the tibialis and soleus of the monkey, rabbit and hare) these drugs initially produced a type of block which had the characteristics of a depolarisation block which was followed by a second phase of block which had the characteristics of competitive block. (A tetanus was not sustained and the block was antagonised by neostigmine).

The observation by Burns and Paton (1951) that successive doses of decamethonium produced a progressively smaller depolarisation in the gracilis muscle of the cat is compatible with the idea of a dual mode of action even on this muscle.

These tests, however, are not conclusive. The justification for the use of the action of anticholinesterase in reversing block as a test for the presence of a (+) - tubocurarine-like competitive block is discussed below. The reversal by  $K^+$  ions of the second phase of block must not be taken as evidence for the presence of a (+) - tubocurarine-like block as it is known that potassium has a direct effect on the muscle fibre /

fibre as well as the end-plate.

More evidence about the nature of the second phase of block caused by decamethonium was obtained when the theory of depolarisation block was tested as a cellular level using microphysiological techniques.

#### Microphysiological Study of Phase II

Thesleff (1955) using the sartorius nerve-muscle preparation from frog with internal and external recording found that acetylcholine, nicotine, decamethonium and suxamethonium all acted in a qualitatively similar way. During the first few minutes there was a block accompanied by a brief depolarisation which subsided spontaneously without the removal of the block. He found that maximal neuromuscular blockade did not occur during this depolarisation phase but only occurred when the membrane potential had returned to about normal. During this block of transmission the end-plate regions of the muscle were insensitive to the depolarising effect of acetylcholine. Thesleff concluded that the neuromuscular block caused by these depolarising drugs was not due to a persistent depolarisation of the end-plate or adjacent /

adjacent muscle membrane but to a decrease in the sensitivity of the end-plate to the transmitter substance. These observations have been repeated on frog muscle by del Castillo and Katz (1957) and have been extended to mammalian muscle by Axelsson and Thesleff (1958) and Thesleff (1958). In all the muscles used it has been found that with decamethonium, and decamethonium-like drugs (acetylcholine, carbachol, nicotine, etc.) the end-plate became repolarised again although the block remained, and that after this there was a phase of block during which the end-plate receptors were desensitised to the further action of acetylcholine, applied ionophoretically (in pulses), at the end-plate region. This phase of desensitisation persisted as long as the blocking drug was maintained at the same concentration.

Differences between the effect of eserine  
on the block of micro and macro preparations.

The observation of a desensitisation to acetylcholine when the second phase of decamethonium block was studied at the cellular level /

level is clearly at variance with the results obtained from the response of the whole muscle studied macropharmacologically. If the end-plate region is completely desensitised how is it that eserine can reverse the block? This is where the main difference between the two techniques becomes apparent: when micro-physiological recordings are made from one fibre the indication of complete block is the failure to record an action potential on the application of acetylcholine, whereas in the whole tissue preparation the indication of block is the failure to produce a contraction on nerve stimulation. This block of contraction, however, does not necessarily mean that all the receptors or indeed all the end-plates are blocked. It may be that there are sufficient unblocked receptors present to produce a contraction due to the lowering of their threshold to nerve stimulation by the presence of the increased acetylcholine. This argument is strengthened by the fact that in many published records purporting to show the reversal by eserine of this second phase of block, the degree of block was less than 100%. In such circumstances there must be /



be receptors which are not occupied by the blocking drug.

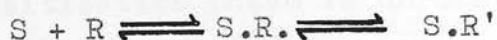
Another explanation of this reversal caused by eserine is that it is due to a repetitive firing of the receptors which have recovered from their desensitised state. The number of such receptors may be large in whole animal experiments, where the effective drug concentration is being reduced steadily, or in isolated preparations after the drug has been washed out.

#### KINETIC THEORY

##### (a) Intermediate complex

Del Castillo and Katz (1957) have tried to explain the various interactions of drugs at the end-plates by the application of kinetic theory. They suggest that the drug receptor reaction which produces depolarisation is a two-step process (by analogy with the cholinesterase - acetylcholine reaction - Augustinsson 1948 and 1949).

This reaction may be represented:-



where S is the substrate, R the hypothetical receptor, S.R.' an intermediate inactive complex, and /

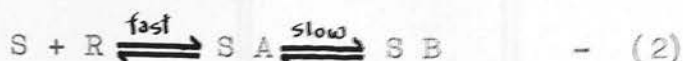
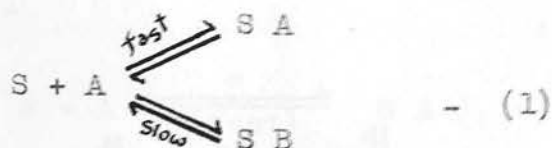
and S.R.' the depolarising complex.

This intermediate complex may be used to explain the blocking action of both (+) - tubocurarine and decamethonium (during the first phase of block). (+) - tubocurarine for instance may be considered to form a reversible intermediate complex (S.R) without proceeding to the next step. Del Castillo and Katz (1957) showed that the rate of development of depolarisation with decamethonium and other depolarising drugs was much slower than that of acetylcholine. This slow passage through the intermediate step may be the reason for the antagonism of decamethonium to the depolarisation caused by fast and powerful agents like acetylcholine and carbachol.

(b) Connection between depolarisation and desensitisation:-

Katz and Thesleff (1957) have considered two main hypothesis to explain the relationship between depolarisation and block: either that the desensitisation phase is unrelated to the depolarisation phase and in fact developes in parallel, or the desensitisation occurs only after the receptors cease to be depolarised, i.e. follows /

follows as a consequence. These two possibilities may be represented by:-

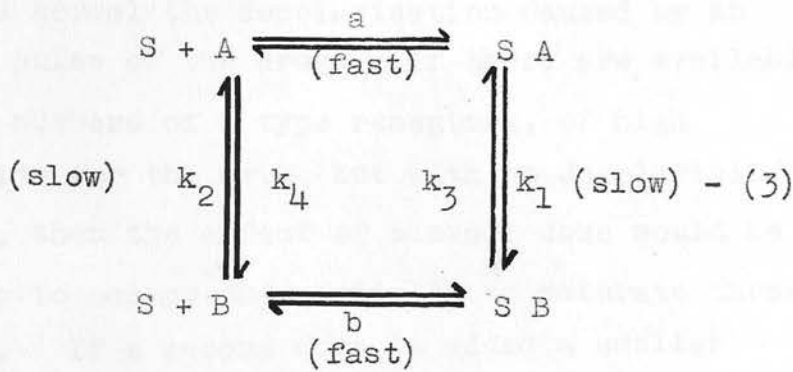


where S is the concentration of the drug, A the free receptors, S A the effective (depolarising) drug-receptor compound, and S B the refractory compound.

Katz and Thesleff pointed out that neither of these simple reactions fit the observed facts. For either of these reactions to be possible the rate of onset of block must be much faster than the rate of recovery. For example for 50% desensitisation the rate of development of block should be twice the rate of recovery. These predictions are clearly at variance with the observed rates: the rate of development of desensitisation was found to be equal to or lower than the rate of recovery. What is required is a reaction in which the recovery process B to A is slowed by the presence of the drug.

An /

An interesting hypothesis they have put forward to satisfy this requirement may be represented:-



where  $a$  and  $b$  are affinity constants,  $k_1$ ,  $k_2$ ,  $k_3$ , and  $k_4$  are the rate constants.

$$\text{Equilibration requires } b/a = \frac{k_1 k_2}{k_3 k_4}$$

This scheme would fit if  $k_1 \gg k_3$ ,  $b \gg a$  and  $b/a > k_1/k_2$ . A feature of this hypothesis is that the free receptors are distributed, even in the absence of the drug, between the states A and B: that is a number of the receptors will be in the refractory form and on account of their very high affinity ( $b/a \gg 1$ ) they will preferentially absorb small quantities of applied acetylcholine.

(c) /

(c) Facilitation

This may explain their observation that a small steady dose of a drug may facilitate beyond normal the depolarisation caused by an added pulse of the drug. If there are available large numbers of B type receptors, of high affinity for the drug, but with no depolarising power, then the effect of a small dose would be mainly to occupy and partially to saturate these sites. If a second dose is added a smaller fraction of the drug molecules would be absorbed by sites B and thus a larger fraction would become available for depolarisation than if the same total dose had been given at once. A different form of facilitation is discussed below under the heading of drugs which have significant activity on the cholinesterase.

Thus, although the available results are not yet accurate enough (quantitatively) to provide a secure basis for kinetic theory they do allow the rejection of certain hypothesis such as (1) and (2). It is not yet possible to decide whether the depolarisation and desensitisation are consecutive or parallel phenomena but one of /

of the more attractive hypothesis (3) includes both of these possibilities and also gives an explanation of one of the forms of facilitation observed.

Anticholinesterase Activity as a Complicating Factor:-

The modes of neuromuscular blocking action described above, however, may be complicated by the ability of the drug to inhibit cholinesterase. Many neuromuscular blocking drugs have this property and this affects greatly their apparent behaviour as neuromuscular blockers. For instance the drug Mytolon (Win 2747) is a powerful inhibitor of cholinesterase:

* Table of pI <sub>50</sub>	<u>Bovine Red Cells</u>	<u>Plasma</u>
Mytolon	6.8	4.5
Eserine	6.6	6.7
Tensilon	5.3	5.6
Neostigmine	6.9	-

(substrate final concentration  $6.7 \times 10^{-4}M$ )

Because it was not reversed by Tensilon it was originally classed as a "depolarising blocking agent". If, however, the effect of Tensilon in reversing the blocking action of curare-like drugs is due to its anti-cholinesterase activity (e.g. Hobbiger 1952, Smith /

\* From Tabachnick, et al. (1958)



Smith et al 1952 and Katz and Thesleff 1957) it is not surprising that it will not reverse the block caused by a drug like Mytolon which is a more potent anticholinesterase than itself. The compounds ambenonium (Mytelase Win 8077) and Win 8078 (Karczmar 1957, Blaber 1960) are other examples of drugs which have both anticholinesterase activity and blocking activity. The reversal by the latter compound of both the block caused by (+) - tubocurarine and that caused by decamethonium may be due to its anticholinesterase activity and to its "curare-like" activity respectively.

It is thus important, when studying the mode of neuromuscular blocking action of a new compound, to find out, by means of invitro experiments, whether it has any significant inhibitory activity on cholinesterase.

#### Mode of Block by nicotine

To conclude the mass of evidence suggests that on the rat diaphragm-phrenic nerve preparation nicotine like other depolarising drugs blocks by a dual mode of action. It appears /

appears (Thesleff 1958) that on this preparation the rate and duration of the initial depolarisation phase are such that the subsequent receptor desensitisation is entirely responsible for the block although the degree and rate of onset of this desensitisation may be graded according to drug concentration used. For instance the duration of the depolarisation due to nicotine in frog muscle is similar to that due to acetylcholine, i.e. 5 minutes (Thesleff 1955) and this may be even less in mammalian tissues where reactions are generally faster (Thesleff 1958). The predominant phase of block may however depend on the particular muscle used. The fact that Burns and Paton (1951) have reported that in the gracilis muscle of the cat an anodal current may even at later stages relieve the neuromuscular block produced by decamethonium suggests that on this muscle desensitisation may develop only slowly and that consequently the duration of the depolarisation period may be prolonged.

The main evidence that the second phase of block is a desensitisation of the receptors, rather /

rather than a "curare-like" reversible form of competition is drawn from the use of microphysiological techniques. It may be concluded that the use of pharmacological tests (e.g. reversal by eserine, acetylcholine, or tetanus) claimed to show that the second phase of block is "curare-like" are open to certain criticisms when they are applied to the classical pharmacological whole muscle preparations in vitro (Taylor 1951) and in vivo (Bacq and Brown 1937), especially when the height of contraction in response to nerve stimulation is taken as the index of blocking activity. The observed "reversal" may be an action either on the percentage of receptors which have not been blocked (desensitised) by the drug or on the percentage of the receptors which have recovered to their sensitive state either after washing out the drug from the bath in vitro or by removal or dilution in in vivo experiments.

#### Conclusion

Thus on the neuromuscular junction the action of nicotine is qualitatively similar to that of the physiological transmitter acetylcholine /

acetylcholine in that it produces: contraction (Page 15), contracture (Page 15), depolarisation (Page 27) and desensitisation (Page 28).

Acetylcholine has been found to stimulate and/or block conduction at the synaptic cleft and the neuromuscular junction. Day (1951) reviewed the activity of such compounds at these sites (i.e. their cholinergic activity). Day also suggested that, at least amongst others of similar activity, the activity might be related to the possession of a partial positive charge on oxygen atom.

Day studied a series of compounds in which the activity of these compounds changed to support his hypothesis. For instance, he showed that substituents on the nitrogen of such groups as  $\alpha$ -amino,  $\alpha$ -hydroxy, and  $\beta$ -hydroxy, which lead to the formation of a positive charge on the nitrogen atom, improved cholinergic activity. However, when the nitrogen atom was substituted with a  $\beta$ -hydroxy group, activity was considerably reduced.

Day (1951 and 1952) has further suggested that the positive charge on the nitrogen atom is important for activity.

## III

Relationship between Chemical Structure  
and Nicotine-like Activity

Beginning with the work of Reid Hunt and Taveau (1911) many compounds related to acetylcholine have been found to stimulate and/or block conduction at the autonomic ganglia and the neuromuscular junction. Hey (1952) reviewed the activity of such compounds at these sites (i.e. their nicotine-like activity - Dale 1914) and suggested that, at least amongst ethers of choline, activity might be related to the possession of a partial positive charge on the oxygen atom.

Hey studied a series of phenylethers of choline and the activity of those compounds seemed to support his hypothesis. For instance he showed that substituents in the nucleus of such groups as m-chloro, m-bromo, and 3:5 dibromo, which lead to the accumulation of a positive charge on the ether oxygen, improved nicotine-like activity, whereas meta and para methyl, and 3:5 dimethyl, groups lowered activity considerably.

Barlow (1955 and 1960) has reviewed subsequent work and suggests that this supports the /

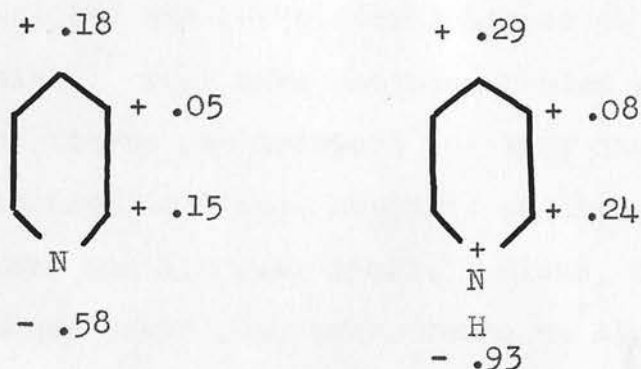
the hypothesis. There is evidence, however, that the partial positive charge need not be sited at an oxygen atom. For instance Welsh and Taub (1951) studied a series of ketoamyl-trimethylammonium salts, on the heart of Venus mercenaria, and still found the 4 keto compound to be highly active although the partial positive charge was on a carbon atom. This compound was found to have the same activity as acetylcholine on the frog.

This idea, that the partial positive charge need not be on an oxygen atom, might account for the activity of nicotine itself.

In the pyridine ring the  $\alpha$  and  $\gamma$  carbon atoms carry a partial positive charge. This is indicated by the great ease of attack by nucleophilic reagents (e.g. by sodamide). The charge distribution, calculated by Longuet-Higgins and Coulson (1947) shows that relative to the 3 carbon atom, the 2 and 4 (and 6) carbon atoms carry an appreciable positive charge. When there is a proton on the pyridine nitrogen the partial positive charge on the 2 and 4 carbon /



carbon atoms is even greater:-



(From Albert 1960)

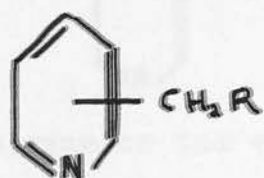
In nicotine the pyridine ring is attached to the  $\beta$  position of the pyrrolidine ring by a single bond from the  $\beta$  position. There is free rotation about this bond and consequently either the 2 or the 4 carbon atoms of the pyridine ring can easily assume the same position relative to the pyrrolidine nitrogen atom.

Taylor (1951) calculated from the dissociation constants of nicotine that it was mainly present as the pyrrolidinium ion at body pH (i.e. with the proton on the pyrrolidine nitrogen). The pyrrolidine nitrogen can therefore be compared with the cationic head in acetylcholine and the partial positive charge on the 2 and 4 position can be compared with the partial positive charge on the ether oxygen of choline phenyl ether.

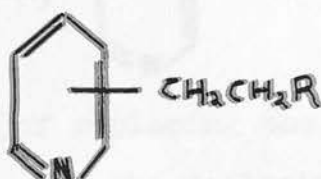
This /

This idea receives some support from the fact that (+) and (-) nicotine appear to be equiactive. They have not been tested on isolated tissue preparations but they have been found to have identical toxicity on the guinea-pig (Hicks and Sinclair 1947). Hicks, Brücke and Heubner (1935), however, found no significant differences between the activity of (+) and (-) nor nicotine on the blood pressure, respiration, vagal and sympathetic ganglia and the heart of cats although there were some slight differences on frog muscle and nerve-muscle preparations.

To test the hypothesis and also to examine the effect of removing the asymmetric centre compounds of the following type have been tested:



Methylene  
linked



Ethylene  
linked

Where R is:-



dimethylamino

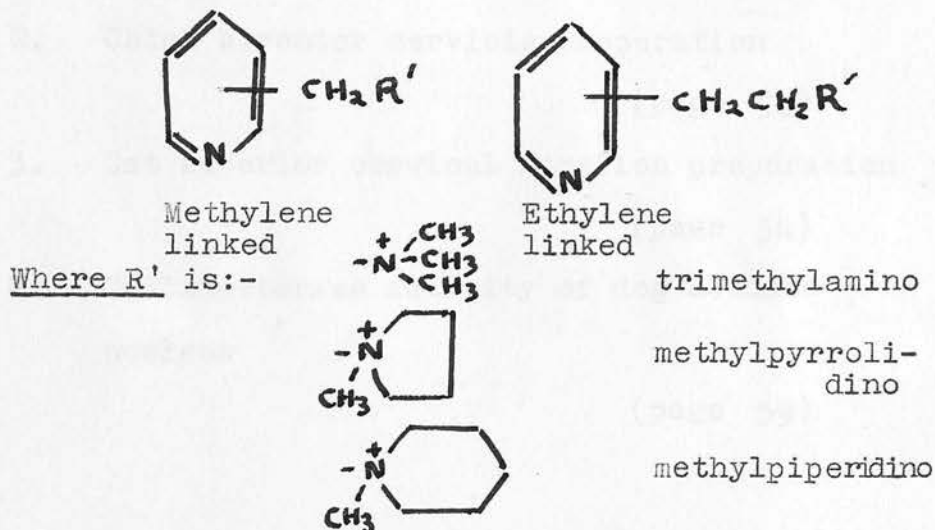


pyrrolidino

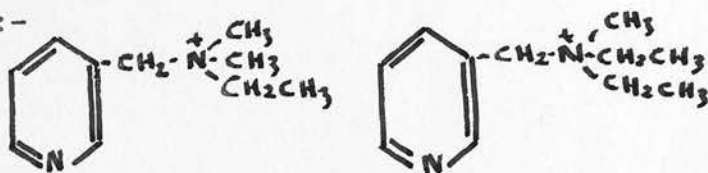


piperidino

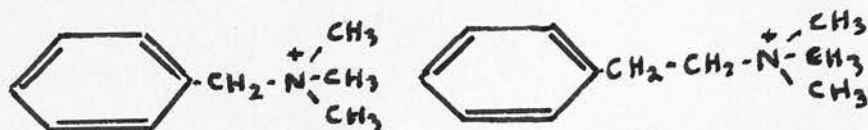
To examine the effect of quaternisation of the substituent nitrogen compounds of the following types have been tested:-



To examine the effect of successively replacing the methyl groups in the cationic head with ethyl groups the following two compounds were tested:-



To examine the effect of replacing the pyridine ring by a benzene ring the following two compounds were tested:-



These compounds were tested on:-

1. Rat Phrenic nerve-diaphragm preparation  
(page 49)
2. Chick biventer cervicis preparation  
(page 51)
3. Cat superior cervical ganglion preparation  
(page 54)
4. Cholinesterase activity of dog caudate  
nucleus  
(page 59)

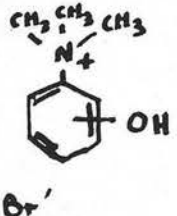
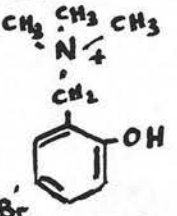
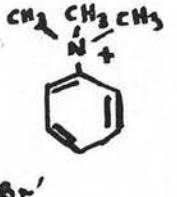
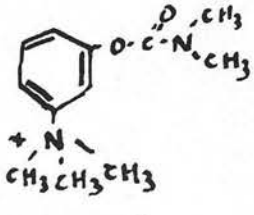
Table 1

Relative Stimulant Activity of phenyltrimethylammonium  
Compounds on Denervated Tibial Anterior  
Muscle of Dog:

on weight basis

m. hydroxyphenyltrimethyl-  
 ammonium bromide = 1.0

(Recalculated from Randall and Lehmann 1950  
 and Randall 1950)

Compound	Relative Activity
	<div style="display: flex; align-items: center; justify-content: center;"> <div style="margin-right: 10px;"> <math>\frac{o}{m}</math>  <math>\frac{h}{m}</math> </div> <div> <p>0.002</p> <p>1.0</p> <p>0.05</p> </div> </div>
	<p>0.5</p>
	<p>4.0</p>
<p>Neostigmine</p>  <p>CH<sub>3</sub>SO<sub>3</sub>CH<sub>3</sub></p>	<p>0.1</p>

Pharmacology of Related Compounds

Some compounds resembling those listed above have been prepared and tested, but not specifically for nicotine-like activity. One group, consisting of phenolic quaternary salts, was studied primarily for their ability to reverse the action of (+) - tubocurarine by Wescoe, Riker, and Brothers (1949), Randal and Lehman (1950) and Randall (1950). The tests did, however, include a comparison of the ability of the compounds to cause a contracture of the denervated anterior tibial muscle of the dog and the results are shown in Table 1 (calculated from Randall 1950).

Similar compounds have also been studied by Kuperman, Gill and Riker (1961) for their effects in potentiating the twitch response of the cat gastrocnemius muscle to nerve stimulation, and as inhibitors of cholinesterase. With one group of compounds (3 hydroxy-, and 4 hydroxy-, trialkylammonium salts) there was a correlation between these two activities; but with a second group there was no such correlation. The dose-effect (potentiation) curves of the second group were /



were much flatter than those of the first group. A third group of compounds only depressed conduction. This block may be a measure of the ability to produce desensitisation, a nicotine-like activity. Phenyltrimethyl ammonium was the drug with the highest blocking activity. It is possible that with the compounds of the intermediate group there exists the ability to produce both effects: anticholinesterase (potentiating) and nicotine-like (blocking) which may well act in opposition to varying extents. This was not the view of the authors who emphasise the possibility of an action at the presynaptic nerve terminal.

Another related substance is Leptodactyline (Glässer 1960, and Erspamer and Glässer 1960). This produced neuromuscular block being more than 28 times as active as nicotine on the cat sciatic-gastrocnemius preparation. It also appeared to have considerable nicotine-like activity, being 40 to 100 times as active as nicotine on the frog rectus, 8 times on the cat nictitating membrane preparation, 2 to 4 times on the blood pressure of a spinal cat and 1 to 2 times as active as nicotine on the leech. Some analogues of leptodactyline have been prepared by these workers but as yet no reference to their activity has appeared in the literature.

## Experimental Section

### METHODS

Although in the introduction, for historical reasons, the activity at the ganglion has been discussed before the activity at the neuromuscular junction, in this section the order is reversed. More work has been done with the compounds on the neuromuscular junction than on the ganglion because the two nerve-muscle preparations used (the rat phrenic nerve-diaphragm and the chick biventer cervicis - see below) are much easier to put up than the ganglion preparation used (cat superior cervical ganglion).

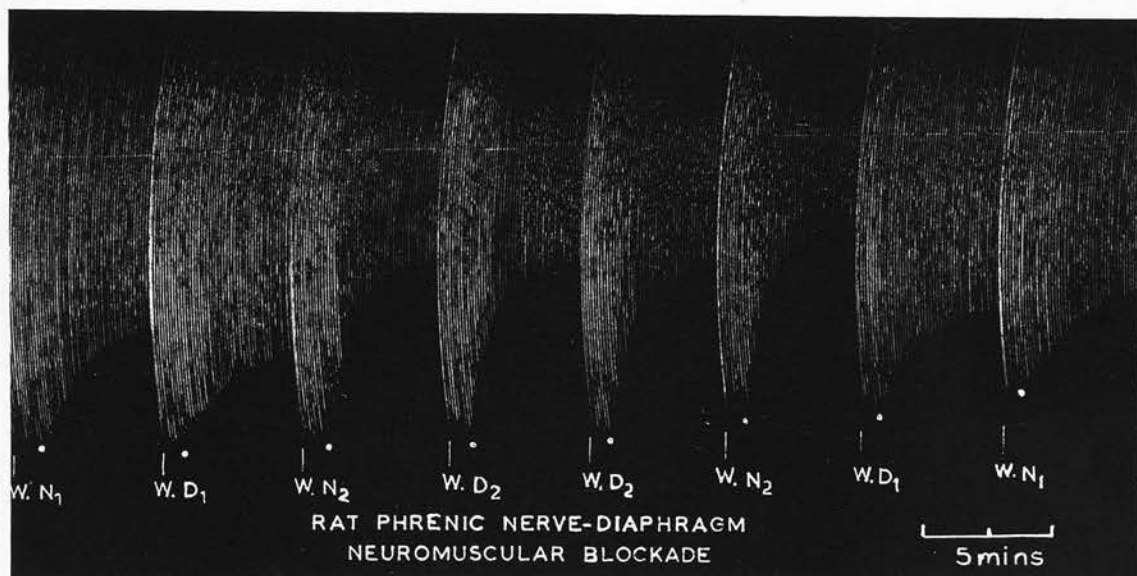
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Figure (1)

Rat Phrenic Nerve-Diaphragm Preparation

Doses added to 25 ml. bath

{ N<sub>2</sub> = 2.5μM Nicotine  
{ N<sub>2</sub> = 5μM "  
{ D<sub>1</sub> = 25μM { γ-pyridyl methyl  
{ D<sub>2</sub> = 50μM { dimethylammonium  
{ W = Wash



Krebs' Solution 37°C. gassed 5% CO<sub>2</sub> and 95% O<sub>2</sub>

Activity at the Neuromuscular Junction:-

I The Rat Phrenic Nerve - Diaphragm Preparation  
(Bülbring 1946)

Rats of either sex weighing between 150 and 250 grams were used. They were killed by a blow on the head and were bled out. The thorax was opened up exposing the left half of the diaphragm and the phrenic nerve. An incision was made through the left abdominal muscles along the costal margin. A fan-shaped strip of diaphragm about 12mm. wide at the costal margin and about 3mm. wide at the tendinous end was cut out. The phrenic nerve was dissected out and was cut as high up as possible leaving about 2.5cms. attached to the muscle. The muscle and nerve during this dissection were kept moistened with Krebs' bicarbonate solution.

The dissected muscle was placed in Krebs' solution in a Petri dish. A thread was tied round the end of the nerve and either a strong linen thread or a wire was tied round the tendinous end of the muscle.

The preparation was mounted on a double hook pushed through the costal margin and was suspended in Krebs' bicarbonate solution in a 25ml. bath at 37°C. The solution was gassed with 5% carbon dioxide and 95% oxygen from a sintered gas distributor.

The /

The nerve was threaded through a pair of platinum electrodes as described by Bülbring and the thread or wire from the muscle was attached to a semi-isometric torsion lever (Condon 1957).

The nerve was stimulated maximally with square wave shocks of 0.75m.secs. duration at a rate of 4 to 6 per minute. The contractions of the muscle were recorded on a smoked drum.

Method:-

Antagonist activity was measured by estimating the molar concentrations of each drug which produced comparable degrees of block. The degree of block was measured by the percentage reduction in the height of contraction of the muscle.

The activity was expressed as an equipotent molar ratio i.e. the number of molecules of the drug which produced the same block as one molecule of the reference standard, nicotine (hydrogen tartrate).

At least two dose levels of each drug were used in each experiment, and the graph of the logarithm of the dose to the response was compared with that for nicotine. The potency ratio was obtained graphically by taking the antilog of the horizontal distance between the nicotine and the drug lines.

If /



If these did not appear to be parallel the value was measured when the block was 50%. Wherever possible a four-point assay was employed and the potency ratio and fiducial limits were calculated by the method for the assay of penicillin described in the British Pharmacopœia 1953. The equipotent molar ratio is the reciprocal of the potency ratio.

All the tertiary compounds were tested on at least two rats; but the quaternary compounds were only tested on one rat.

The drugs were all made up in isotonic saline and were added to the bath from a blow-out pipette. They were left in the bath for 5 to 10 minutes depending on the rate of development of block, and were washed out by emptying and filling the bath three times with fresh Krebs' solution. The preparation was allowed to recover until the original height of contraction was restored before the next dose was given.

## II The Chicken Biventer Cervicis Preparation (Ginsborg & Warriner 1960)

Chickens (500-200 gms. Rhode Island Reds) were anaesthetised with ether. The back of the neck was plucked and the skin incised along the midline from the base of the neck to the skull, exposing the two biventer cervicis muscles on either side of the midline /

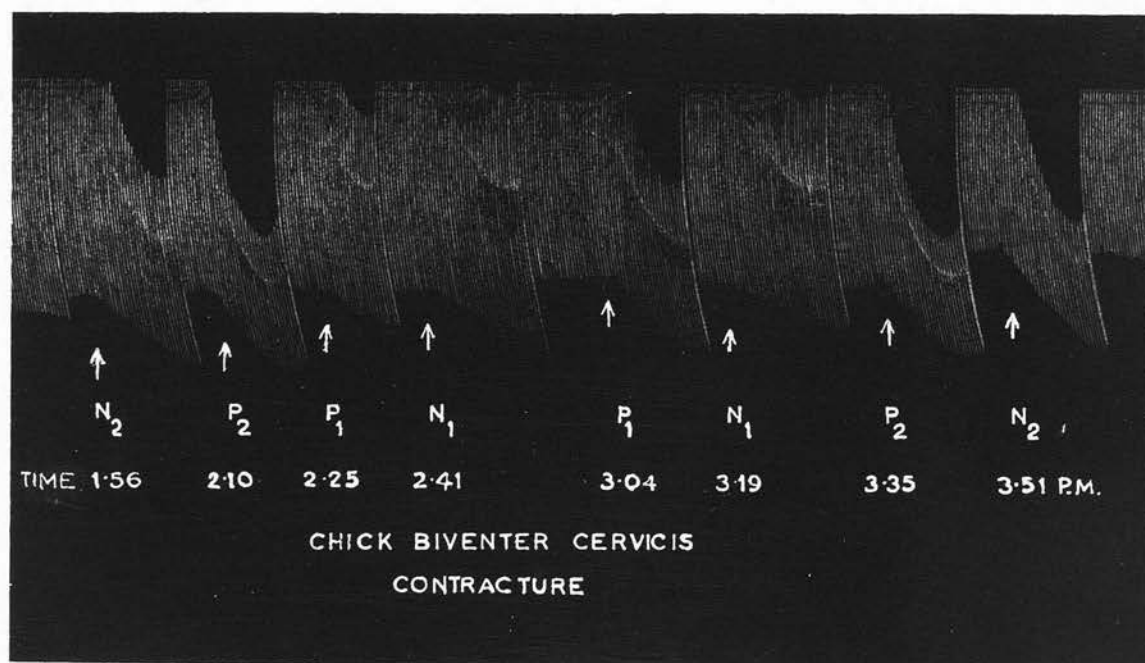


Figure (2)

Chick Biventer Cervicis Preparation

Doses added to 15 ml. bath

(N<sub>1</sub> = 40nM Nicotine  
{ N<sub>2</sub> = 50nM "  
{ P<sub>1</sub> = 2μM β-pyridyl methyl piperidine  
(P<sub>2</sub> = 2.5 μM " " "



Krebs' Solution 37°C. gassed 5% CO<sub>2</sub> and 95% O<sub>2</sub>

midline and immediately below the skin.

A thread was tied round the upper belly of one of the muscles which was cut free from its attachment to the skull. The tendon and the muscle were dissected free and were removed with the lower tendon which attaches the muscle to the supraspinous ligament.

A loop of thread was tied round the lower tendon so that the muscle could be hooked to the end of a rod which acted as a gas inlet. The upper thread was passed through the same electrode as was used in the rat diaphragm preparation, and was attached to a light semi-isometric lever. The preparation was placed in a 15 ml. bath containing Krebs' solution at 37°C. gassed with 5% carbon dioxide and 95% oxygen.

The electrode was lowered until it was in contact with the tendon surrounding the nerve. The nerve was stimulated maximally with square wave shocks of 0.75 m.secs. duration at a rate of 4 to 6 per minute. The contractions of the muscle were recorded on a smoked drum.

Method:-

Agonist activity (i.e. the ability to produce /

produce contracture) was measured by estimating the molar concentrations of each drug which produced comparable degrees of contracture. The degree of contracture was measured as the number of mms. rise in height of the base-line on the drum.

The activity was expressed as an equipotent molar ratio, i.e. the number of molecules of the drug which produced the same degree of contracture as one molecule of nicotine.

The degree of contracture was measured for each drug at two dose levels and the response was plotted against the logarithm of the dose. The potency ratio was determined exactly as in the experiments on the rat diaphragm, and in a number of experiments a full four-point assay was performed. The equipotent molar ratio is the reciprocal of the potency ratio.

All the tertiary compounds were tested on at least two chickens, but the quaternary compounds were only tested on one animal.

The /

The drugs were made up in isotonic saline and were added to the bath from a blow-out pipette. They were left in the bath until the degree of contracture had reached a maximum for the dose given. After washing out three times the muscle was allowed to recover for the same length of time as the drug was in the bath.

#### Activity at Ganglia:-

##### The Cat Superior Cervical Ganglion Preparation (Paton and Perry 1953 and Trendelenburg 1954)

The cats used were of either sex and weighed from 2 to 4 Kg. They were anaesthetised with ether in a box and were placed on a warmed table.

The trachea was cannulated and was connected by a short tube to a Wolff bottle containing ether. The right femoral vein was exposed and cannulated and 80mg./Kg. chloralose was given slowly in a 1% solution of saline at 38°C.

The cat's head was immobilised by securing the snout to a transverse bar placed between the jaws: this was firmly clamped to the table.

A thread was sewn into the right nictitating membrane. This was passed under a pulley and attached to a frontal writing lever. In a number of cats the responses from both the right and the left membranes were recorded.

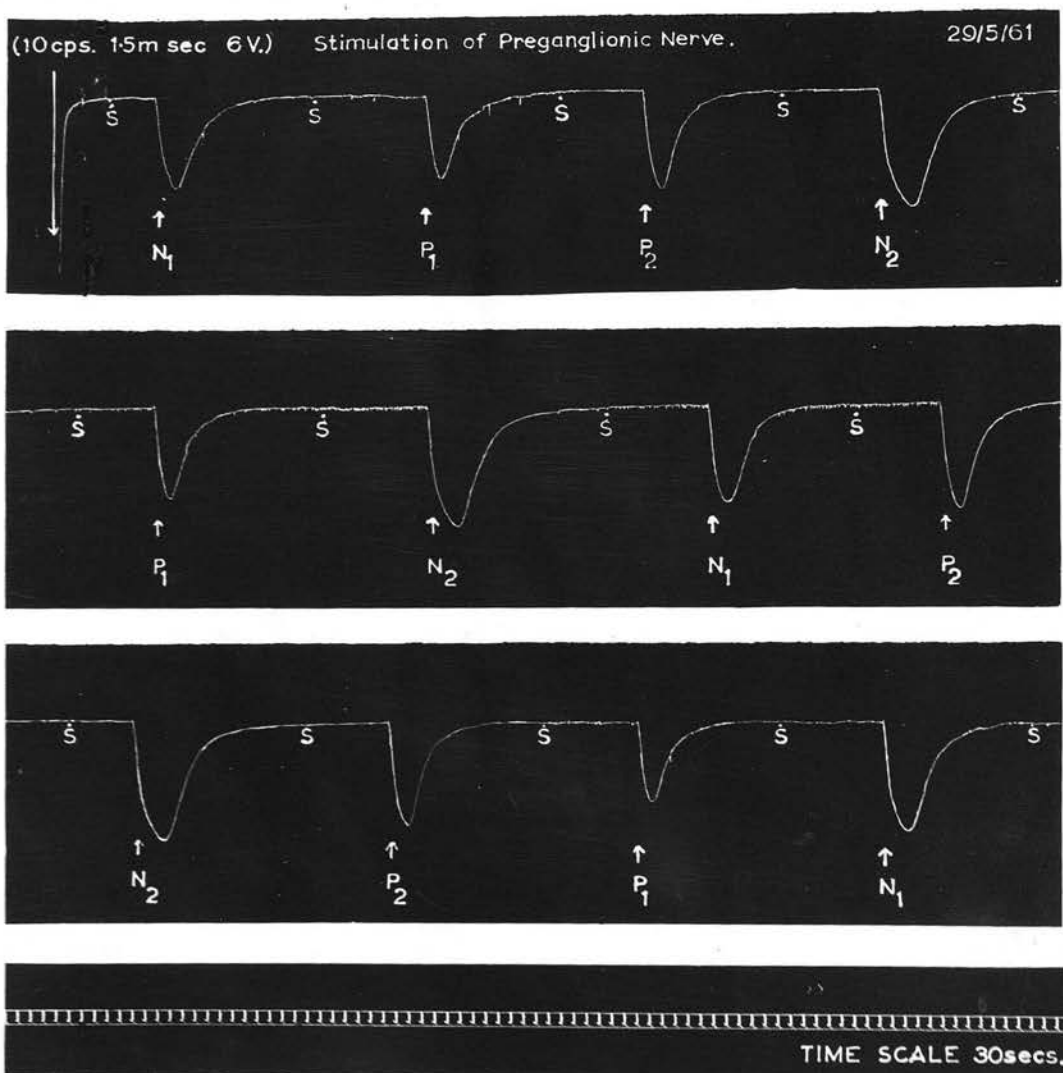
A length of the common carotid artery up to the external carotid was exposed. All the blood vessels excluding the lingual artery and the vessels supplying the superior cervical ganglia were cut between two ligatures.

If the lingual artery was of sufficient diameter a Luer-lock syringe needle, adapted as a cannula, was inserted into it and the external and internal carotid arteries were tied off. A remote control bulldog clip was applied to the lingual artery between the needle and the carotid artery before cannulation. The needle was previously filled with saline and both the needle and the bulldog clip were securely clamped to the table.

If the lingual artery was not big enough to be cannulated the external carotid artery was cannulated retrogradely instead in a similar manner to that described above. This time the internal /

Figure (3)

CAT SUPERIOR CERVICAL GANGLION BLOCKADE



injections Retrogradely into External Carotid Artery  
in 0.1ml Saline

$N_1 = 100\text{nM}$   
 $N_2 = 150\text{nM}$  } NICOTINE  
 $P_1 = 200\text{nM}$   
 $P_2 = 300\text{nM}$  } c1ccc(cc1)CN2CCCCC2 diHBr  
 S = 0.1ml Saline

4.0Kg ♀ Cat.  
 320mg Chloralose iv.

internal carotid and lingual arteries were tied off.

A length of the right preganglionic sympathetic nerve was separated from the vagus and was cut. The central end was laid on a pair of shielded platinum electrodes.

A blood pressure record was normally taken by means of a mercury manometer with a cannula in the left femoral artery. Saline was used as the connecting bridge and sufficient heparin was added to the saline at the tip of the cannula to prevent clotting.

During the completion of the dissection small quantities of saline were injected by means of a tuberculin syringe into the needle in order to prevent clotting.

The nerve was stimulated maximally with square wave shocks of 0.75m.secs. duration at a rate of 10 per second and the contraction of the right nictitating membrane was recorded on a smoked drum.

Method:-

- (i) Antagonist activity (i.e. the ability to produce ganglion-block) was measured by estimating the molar concentrations of each drug which produced /



produced comparable degrees of block. The ganglion-block was measured as the relaxation of the nictitating membrane (in mms.) during maximal preganglionic nerve stimulation.

The activity was expressed as an equipotent molar ratio (i.e. the number of molecules of the drug which produced the same block as one molecule of nicotine.)

The equipotent molar ratio was determined exactly as in the experiments on the rat diaphragm. Four point assays were performed with a number of drugs and the activity of the others was determined graphically. Most of the compounds were tested on two cats, a few on three or four, and a few on only one cat.

$M/10$  stock solutions of the drugs in saline were stored at  $-20^{\circ}\text{C}$ . Doses were given close intra-arterially to the ganglion in 0.1 ml. from /

from a tuberculin syringe containing an appropriate dilution of the stock solution.

Before each injection the needle was filled with the drug solution to make sure no air bubbles were injected. After the effects of a dose had almost passed off an injection of 0.2ml. of saline was given. If this produced a response, indicating that the dose of drug had not all gone in, a further 0.2 ml. saline was injected.

Doses producing between 10 and 80% block were given and a time cycle was chosen which allowed complete recovery from the largest dose used. This period varied from 10 to 30 minutes.

- (ii) Agonist activity was studied by observing rises in arterial blood pressure and/or contractions of the unstimulated nictitating membrane.

Blood pressure records were taken /

taken in almost all the experiments with the cat superior cervical ganglion. These only gave rough indications of pressor or depressor activity.

In a few instances the electrical stimulation of the preganglionic nerve was switched off and the ganglion stimulant activity was measured by observing the contraction (in mms.) of the nictitating membrane produced by doses of the drugs.

An equipotent molar ratio was evaluated either by a four-point assay, or, more usually, graphically as in the experiments on the rat diaphragm.

#### Activity on Cholinesterase

##### Enzyme Source:-

The Cholinesterase used was the enzyme present in dog caudate nucleus which is regarded as an acetylcholinesterase. (Mendel et al 1943; Sturge and Whitaker 1950).

An /

An acetone powder was prepared from the caudate nuclei of five dogs which had been anaesthetised with thiopentone and bled out. The brains were removed within 30 minutes of death and the caudate nuclei were removed and stored at  $-20^{\circ}\text{C}$ . overnight. The nuclei were homogenised in ice-cold acetone with an "Atomix" blender. The acetone was filtered off and the residual powder was washed several times with cold acetone, dried in vacuum over calcium chloride, and stored at room temperature over calcium chloride.

When required for use a weighed portion of this powder was homogenised by hand and was suspended in Krebs' bicarbonate buffer solution.

Method:- (Ammon 1934)

(a) Control Experiments

The rate of hydrolysis of the substrate acetylcholine, was followed manometrically at constant volume and at  $37^{\circ}\text{C}$ . by observing the evolution of carbon dioxide from Krebs' bicarbonate buffer by the acetic acid produced.

The reaction was performed in an atmosphere of 5% carbon dioxide 95% nitrogen in a constant volume manometer. /

manometer. This instrument is a modification of the "blood-gas manometer" described by Barcroft and Haldane (1902).

Control experiments were first performed showing the hydrolysis of acetylcholine in the absence of any inhibitor. The effects of substrate concentration on the rate of hydrolysis were studied using various amounts of acetone powder. The purpose of these experiments was to establish the minimum amount of acetone powder which could satisfactorily be used and the degree of saturation of the enzyme produced by particular concentrations of the substrate.

Flasks were prepared as follows:-

- I Thermobarometer - containing water only.
- II Blank - containing buffer, acetone powder, but no substrate.
- III - XII Test flasks containing buffer, acetone powder and substrate.

Such /

Table 2

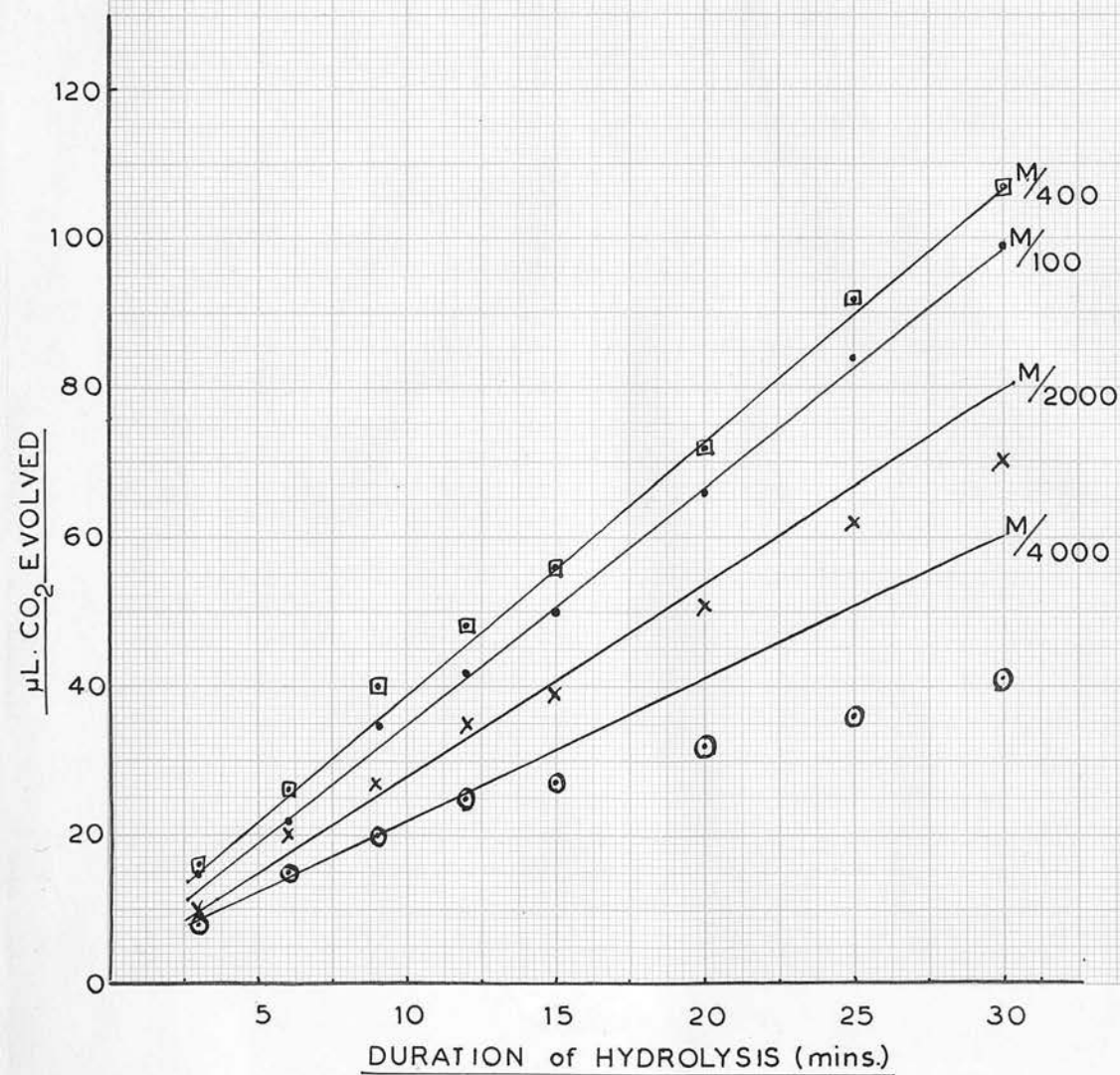
EXPERIMENT 18/10/60. 6mg ACETONE POWDER/FLASK. SUBSTRATE CONCENTRATION FROM M/100 - M/4000  
 Gassed 10 minutes with 5% CO<sub>2</sub> 95% N<sub>2</sub>. Equilibrated 15 minutes at 37°C. Substrate made up in KREBS' bicarbonate buffer

FLASK NUMBER	THERMO-BAROMETER (new 5)	BLANK (old 1)	M/100 Ach (old 2)	M/200 Ach (old 3)	M/400 Ach (old 6)	M/1000 Ach (old 7)	M/2000 Ach (old 8)	M/4000 Ach (old 9)
MAIN FLASK 1 Suspension of powder 2 Buffer	6 mls. Water	0.6 mls. 5 mls. Krebs'	0.6 mls. 3 mls. Krebs'	0.6 mls. 3.2 mls. Krebs'	0.6 mls. 3.3 mls. Krebs'	0.6 mls. 4.8 mls. Krebs'	0.6 mls. 7.0 mls. Krebs'	0.6 mls. 7.2 mls. Krebs'
SIDE BULB Substrate	—	0.4 ml. Water	0.4 ml. M/10 Ach.	0.2 ml. M/10 Ach.	0.1 ml. M/10 Ach.	0.6 ml. M/100 Ach.	0.4 ml. M/100 Ach.	0.2 ml. M/100 Ach.
TOTAL VOLUME	6 mls.	6 mls.	4 mls.	4 mls.	4 mls.	6 mls.	8 mls.	8 mls.
ABSOLUTE AMOUNT Ach	—	—	40 μM	20 μM	10 μM	6 μM	4 μM	2 μM
THEORETICAL EVOLUTION of CO <sub>2</sub>			896 μl	448 μl	224 μl	134.4 μl	89.6 μl	44.8 μl
FLASK CONSTANT K CO <sub>2</sub>		2.02	1.83	1.97	2.01	1.90	1.94	1.77
INITIAL READING	-48	-64	-73	-84	-87	-50	-86	-36
3 Minutes	-49 -1	-67 -2 -4	-68*66 +11+15	-79 +6 +12+16	-82 +6 +12+16	-45 +6 +11+15	-84 +3 +6 +10	-35 +2 +4 +8
6 Minutes	-49 -1	-67 -2 -4	-64 +10 +18+22	-74 +11 +22+26	-77 +11 +22+26	-39 +12 +23+27	-79 +8 +16+20	-31 +6 +11 +15
9 Minutes	-49 -1	-67 -2 -4	-57 +17 +31+35	-68 +17 +33+37	-70 +18 +36+40	-33 +18 +34+38	-75 +12 +23+27	-28 +9 +16 +20
12 Minutes	-49 -1	-66 -1 -2	-52 +22 +40+42	-62 +23 +45+47	-65 +23 +46+48	-28 +23 +44+46	-70 +17 +33+35	-24 +13 +23 +25
15 Minutes	-48 -0	-65 -1 -2	-47 +26 +48+50	-57 +27 +53+55	-60 +27 +54+56	-24 +26 +50+52	-67 +19 +37+39	-22 +14 +25 +27
20 Minutes	-48 -0	-65 -1 -2	-38 +35 +64+66	-48 +36 +71+73	-52 +35 +70+72	-17 +33 +63+65	-61 +25 +49+51	-19 +17 +30 +32
25 Minutes	-49 -1	-66 -1 -2	-29 +45 +82+84	-39 +46 +91+93	-43 +43 +90+92	-9 +41 +78+80	-56 +31 +60+62	-18 +19 +34 +36
30 Minutes	-49 -1	-66 -1 -2	-21 +53 +97 +99	-31 +54 +106+108	-36 +52 +105+107	-3 +47 +89+91	-52 +35 +68 +70	-15 +22 +39 +41
From Graph Evolution CO <sub>2</sub> 5 -15 mins.			31	34	34	33	26	18
X3 ≡ 30 mins.			93	102	102	99	78	54



GRAPH I

EXPERIMENT 18/10/60. 6mg. ACETONE POWDER  
GRAPH of CO<sub>2</sub> EVOLVED (μL) / DURATION of HYDROLYSIS (mins.)





Such an experiment is shown in Table 2 and Graph I. The rate of evolution is measured from the initial slope (5 to 15 minutes).

Notes:-

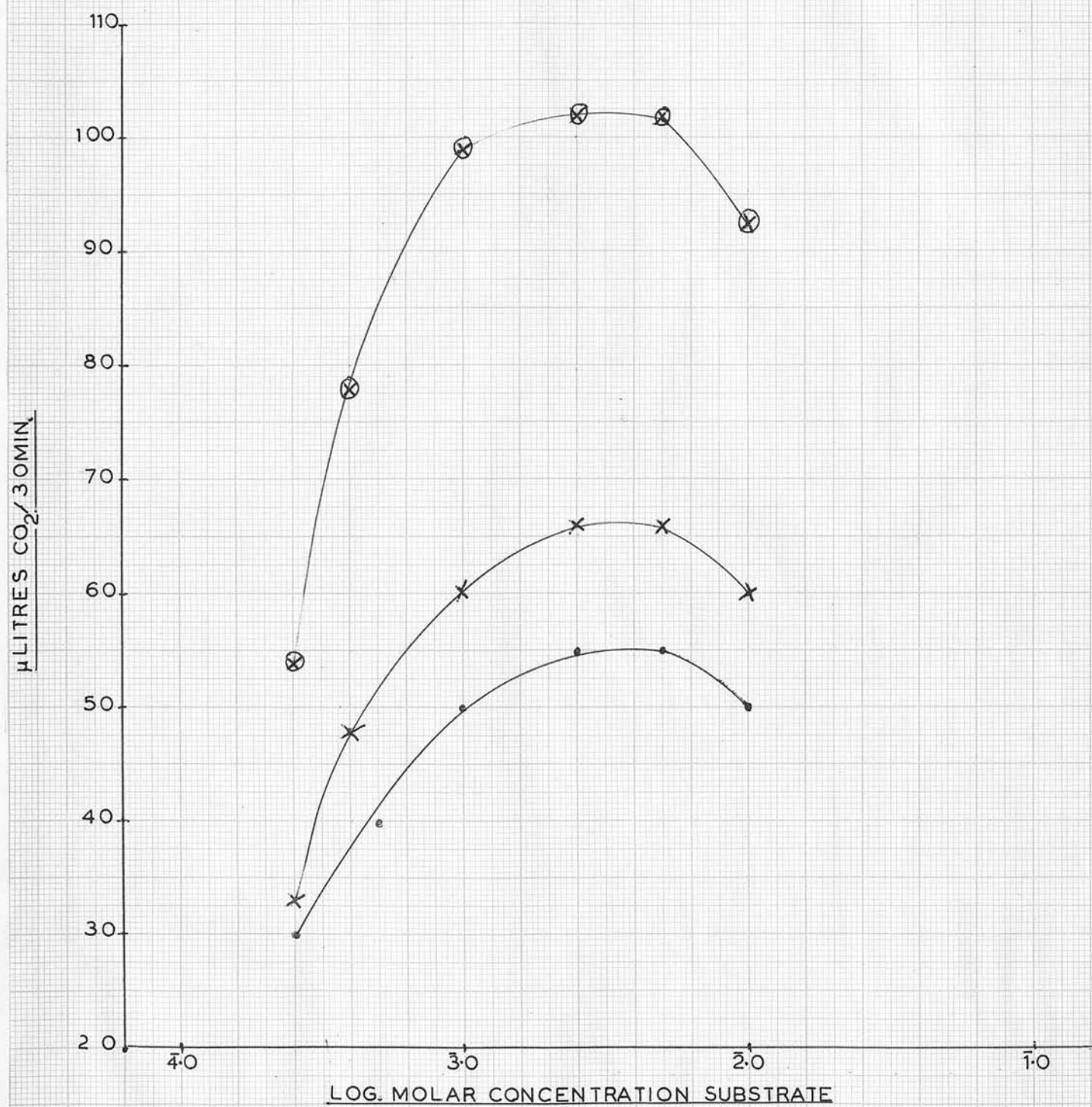
1. It is essential to make up the acetylcholine in buffer immediately before used. If it is made up in distilled water the solution is distinctly acid and will cause an evolution of carbon dioxide from the Krebs' solution on tipping.
2. The flasks were gassed with 5% carbon dioxide, 95% nitrogen for 5 to 10 minutes and were equilibrated at 37°C. for 15 minutes before tipping.
3. Additional blanks containing acetone powder and buffer but no substrate were added if the blanks appeared to be erratic.
4. The total amount of substrate present was such that the substrate would not be all exhausted.

Table 3

Relationship between Rate of hydrolysis  
and substrate concentration

Enzyme Tissue/ flask	µl. Carbon dioxide evolved /30 mins. at different substrate concentrations					
	0.01M	0.005M	0.0025M	0.001M	0.005	0.00025
3 mg	50	55	55	50	40	30
4 mg	60	66	66	60	48	33
6 mg	93	102	102	99	78	54

GRAPH OF SUBSTRATE CONCENTRATION  
/RATE OF HYDROLYSIS ( $\mu$ Litre  $\text{CO}_2$ /30 mins.)



⊗ = 6mg Tissue

X = 4mg Tissue

• = 3mg Tissue

The results of these experiments are shown in Table 3 and Graph II.

(b) Test of Inhibitors:

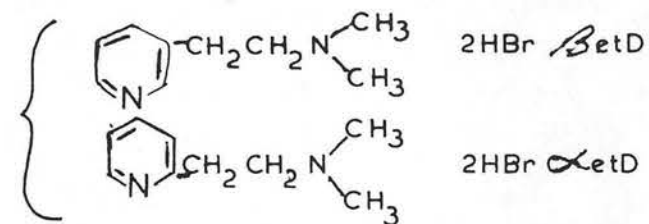
From these results it was decided to test the inhibitors on the hydrolysis of  $10^{-3}$  acetylcholine by 4 mgs. of acetone powder. The total volume of fluid in each flask was 6 mls.

The concentration of inhibitor which produced 50% inhibition of the hydrolysis of this concentration of acetylcholine was determined, and log of the reciprocal of this, the  $pi_{50}$  was used as an index of inhibitory activity.

The flasks were set up as follows:-

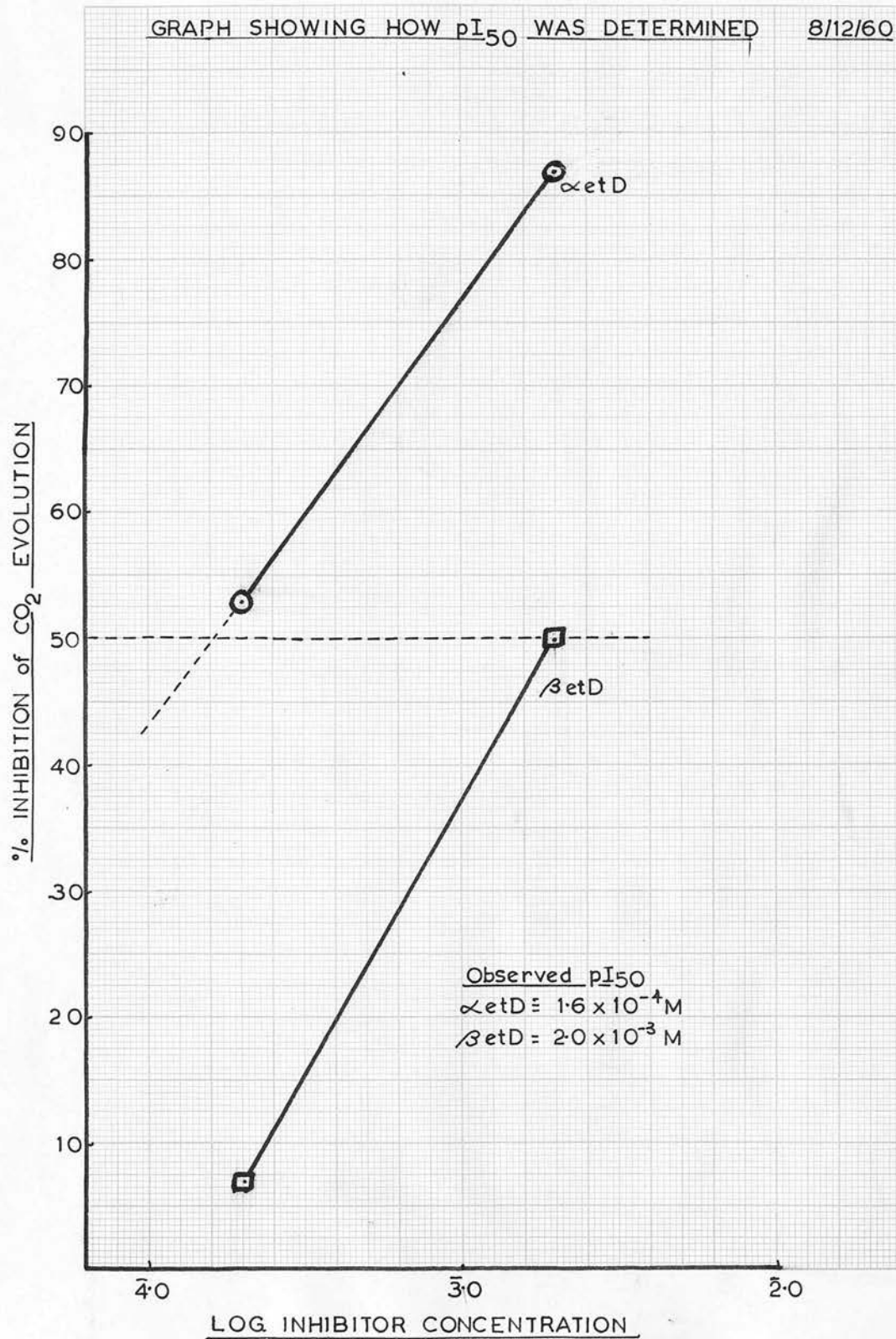
- I Thermobarometer - containing water only.
- II Blank - containing buffer, acetone powder, but no substrate.
- III /

EXPERIMENT 8/12/60. 4mg. ACETONE POWDER / FLASK. INHIBITOR ACTIVITY  
 Gassed 10 minutes with 5% CO<sub>2</sub> 95% N<sub>2</sub>. Equilibrated 15 minutes at 37°C.  
 Substrate made up in KREBS bicarbonate buffer.  
 M/1000 Acetylcholine in each flask.— Total Volume 6mls — Maximum evolution of  
 134.4 μL CO<sub>2</sub> / Flask.



FLASK NUMBER	THERM-BAROMETER (old 5)	CONTROL BLANK (old 1)	INHIBITOR BLANK (old 3)	NO INHIBITOR (old 2)	$\alpha$ etD M/5000 (old 8)	$\beta$ etD M/5000 (old 9)	NO INHIBITOR (old 10)	$\alpha$ etD M/500 (old 11)	$\beta$ etD M/500 (old 12)	$\beta$ etD M/500 (old 14)
MAIN FLASK										
1. Suspension of powder	—	0.4 mls.	0.4 mls.	0.4 mls.	0.4 mls.	0.4 mls.	0.4 mls.	0.4 mls.	0.4 mls.	0.4 mls.
2. Buffer	—	5.3 ml. Krebs'	4.7 ml. Krebs'	5.3 ml. Krebs'	4.7 ml. Krebs'	4.7 ml. Krebs'	5.3 ml. Krebs'	4.7 ml. Krebs'	4.7 ml. Krebs'	4.7 ml. Krebs'
3. Inhibitor	6ml. water	—	0.6 ml. M/500 $\alpha$ etD	—	0.6 ml. M/500 $\alpha$ etD	0.6 ml. M/500 $\beta$ etD	—	0.6 ml. M/50 $\alpha$ etD	0.6 ml. M/50 $\beta$ etD	0.6 ml. M/50 $\beta$ etD
SIDE BULB Substrate	—	0.3 ml. water	0.3 ml. water	0.3 ml. M/50 Ach.	0.3 ml. M/50 Ach.	0.3 ml. M/50 Ach.	0.3 ml. M/50 Ach.	0.3 ml. M/50 Ach.	0.3 ml. M/50 Ach.	0.3 ml. M/50 Ach.
FLASK CONSTANT K.CO <sub>2</sub>	—	2.02	1.91	1.78	2.00	1.83	1.55	1.88	2.09	1.91
INITIAL READING	-50	-76	-86	-87	-65	-54	-79	-55	-66	-77
3 Minutes	-49 +1	-76 -1 -2	-87 -2 -4	-87 +1 +2 +4	-63 +1 +2 +6	-52 +1 +2 +6	-77 +1 +2 +4	-54 +0 +0 +4	-65 +0 +0 +4	-76 +0 +0 +4
6 Minutes	-50 +0	-75 +1 +2	-86 +0 +0	-83 +4 +7 +5	-61 +4 +8 +8	-50 +4 +7 +7	-74 +5 +8 +6	-53 +2 +4 +4	-64 +2 +4 +4	-75 +2 +4 +4
10 Minutes	-49 +1	-76 -1 -2	-84 +1 +2	-80 +6 +11 +13	-59 +5 +10 +8	-46 +7 +13 +11	-69 +9 +14 +16	-52 +2 +4 +2	-62 +3 +6 +4	-72 +4 +8 +6
15 Minutes	-50 +0	-75 +1 +2	-85 +1 +2	-76 +11 +20 +18	-58 +7 +14 +12	-43 +11 +20 +18	-65 +14 +22 +20	-51 +4 +8 +6	-59 +7 +15 +13	-70 +7 +13 +11
20 Minutes	-50 +0	-75 +1 +2	-85 +1 +2	-72 +15 +27 +25	-57 +8 +16 +14	-41 +13 +24 +22	-60 +19 +29 +27	-51 +4 +8 +6	-58 +8 +17 +15	-68 +9 +17 +15
25 Minutes	-51 -1	-76 +1 +2	-84 +3 +6	-69 +19 +34 +32	-56 +10 +20 +14	-37 +18 +33 +27	-56 +24 +37 +35	-52 +4 +8 +2	-56 +11 23 +17	-66 -12 +23 +17
30 Minutes	-52 -2	-77 +1 +2	-86 +2 +4	-65 +24 +43 +41	-54 +13 +26 +22	-34 +22 +40 +36	-52 +29 +45 +43	-51 +6 +11 +7	-54 +11 +29 +25	-64 +15 +29 +25
From Graph Evolution CO <sub>2</sub> 5-15mins				15	7	14		2	7.5	7.5
X 3 $\equiv$ 30mins				45	21	42		6	22.5	22.5
% Inhibition				—	53%	7%		87%	50%	50%





- III Control - containing buffer, acetone powder, and substrate.
- IV Inhibitor Blank - containing buffer, acetone powder, inhibitor, but no substrate.
- V Inhibitor Test - containing buffer, acetone powder, inhibitor and substrate.

Notes:-

1. The concentration of inhibitor used in the inhibitor blank was the highest used in the test flasks.
2. The inhibitor was placed in the main flask with the acetone powder and buffer and the substrate in the side bulb.
3. Fresh  $M/10$  solutions of drugs in deionised water were prepared for these experiments.

A typical experiment is shown in Table 4 and Graph III shows how the  $pI_{50}$  was obtained from the initial rates of evolution expressed as /

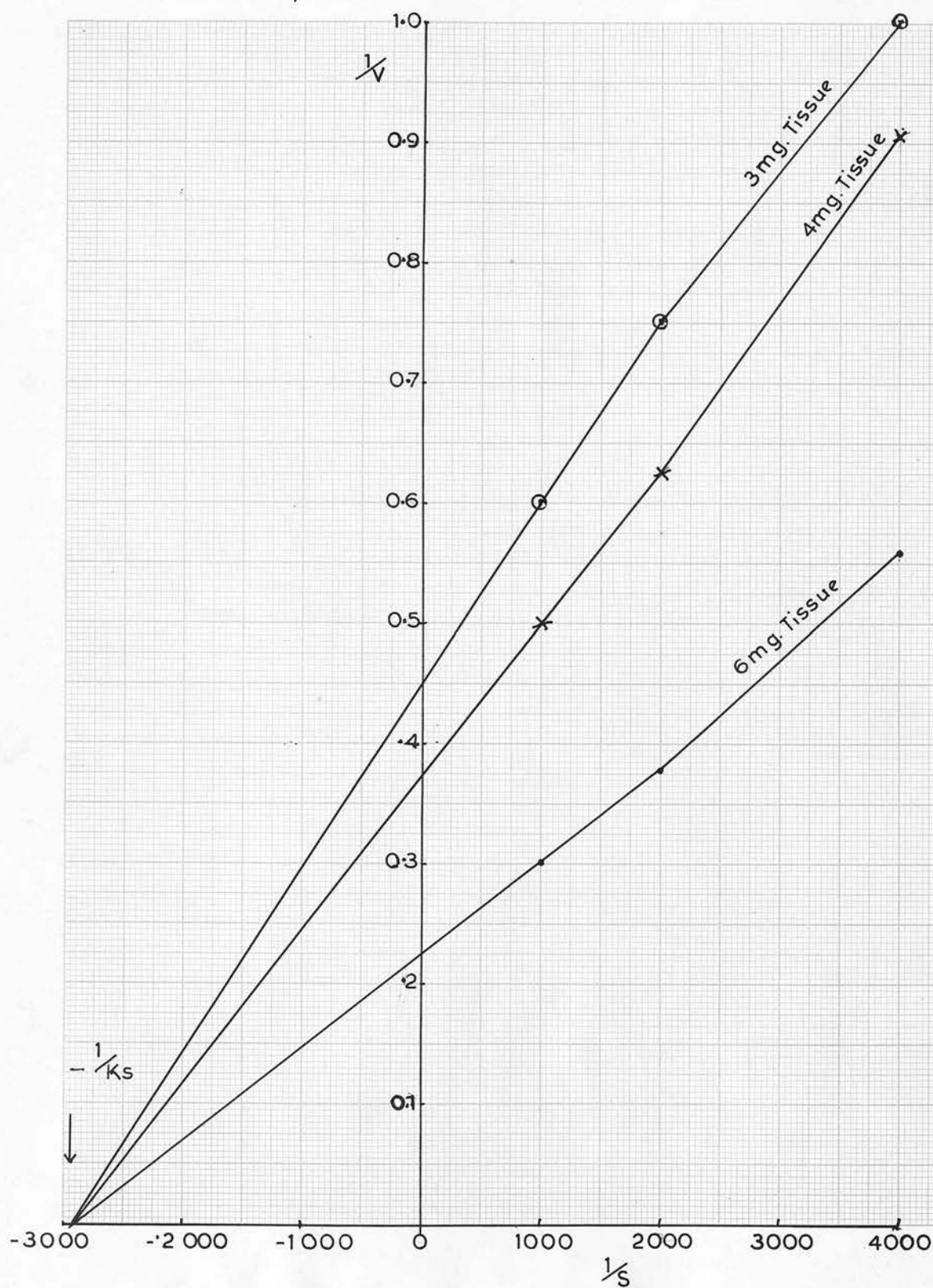


Table 5Determination of Ks

Tissue	Molar conc. Ach. S	1/s	Rate of hydrolysis 1 CO <sub>2</sub> /min	1/v
3 mg	0.001	1000	1.7	0.60
	0.0005	2000	1.3	0.75
	0.00025	4000	1.0	1.00
4 mg	0.001	1000	2.0	0.50
	0.0005	2000	1.6	0.63
	0.00025	4000	1.1	0.91
6 mg	0.001	1000	3.3	0.30
	0.0005	2000	2.6	0.38
	0.00025	4000	1.8	0.56

# GRAPH IV

GRAPH of  $\frac{1}{V} / \frac{1}{S}$  : DETERMINATION of  $K_s$  GRAPH.



as a percentage of the control rate  
(non-inhibited).

Comment:-

After all the experiments had been done it was realised that this was not a very good way of expressing inhibitory activity. The value of  $pI_{50}$  will vary with the substrate concentration used. The relationship is given by:-

$$1 + \frac{S}{K_s} = \frac{I}{K_i} \quad - \text{Equation (1)}$$

where S = substrate concentration

I = inhibitor concentration

$K_s$  = dissociation constant of

Enzyme - substrate

complex

$K_i$  = dissociation constant

of the Enzyme -

inhibitor complex.

It would be much better to measure the  $K_i$  which will be a true constant (at  $37^{\circ}\text{C}$ ). Fortunately this can actually be estimated because  $K_s$  (the Michaelis Constant) can be obtained from the results in Table 5. Graph IV shows how  $K_s$  is obtained from these results by the method of Lineweaver and Burk (1934).

The /

The intercept gives a value for

$$-1/K_s = -2900$$

$$\text{Hence } K_s = 3.45 \times 10^{-4}$$

From equation (1)

$$\begin{aligned} -\log K_i &= pI_{50} + \log (1 + S/K_s) \\ &= pI_{50} + \log (1 + 10^{-3}/3.45 \times 10^{-4}) \\ &= pI_{50} + \log 3.9 \end{aligned}$$

∴ In these experiments

$$-\log K_i = pI_{50} + 0.59$$

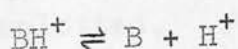
A much simpler method of finding  $K_i$  is that of Dixon (1953) in which the inhibition is studied with a series of inhibitor concentrations at two substrate concentrations and  $1/v$  is plotted against  $i$  ( $v$  = rate of hydrolysis,  $i$  = inhibitor concentration). The intersect of these two lines gives  $-K_i$  directly.

### Measurement of Dissociation Constants

The pKa values of the nicotine analogues were found by potentiometric titration after the method of Goldacre and Albert (1943).

10 mls. of an  $M/100$  solution of the hydrobromide or dihydrobromide of the drug in deionised water was titrated with standard alkali by means of a glass electrode placed in the solution and connected with a Marconi pH Meter (Type TF511D). The other half of the cell was a calomel electrode connected to the solution by a potassium chloride bridge. The solution was continuously stirred by a quickfit compressed air-driven stirring rod (MU8/O) and was maintained at  $25^{\circ} \pm 0.1^{\circ}\text{C}$ . in a water bath.

The dissociation of the salt is:-



$$\text{whence } K_a = \frac{[\text{H}^+][\text{B}]}{[\text{BH}^+]} \quad \left( \begin{array}{l} \text{neglecting} \\ \text{activity} \\ \text{coefficients} \end{array} \right)$$

and at half neutralisation

$$[\text{B}] = [\text{BH}^+]$$

$$\therefore \text{at half neutralisation } K_a = [\text{H}^+]$$

$$\therefore \text{pKa} = \text{pH}$$

The pKa values of the dihydrobromides were therefore /

therefore taken as the pH of the solution after the addition of 0.5 and 1.5 mls. of  $N/10$  sodium hydroxide solution. In the case of the mono-quaternary compounds there can only be one pKa value.

In two instances the hydrobromides were not available: nicotine monomethiodide and nicotine hydrogen tartrate.

The nicotine monomethiodide was titrated against an equivalent amount of standard hydrochloric acid. Nicotine hydrogen tartrate was converted to the base by passing it down a column of I.R.A. 400. The effluent was titrated against standard hydrochloric acid and the pH curve was plotted. An equivalent of standard sodium hydroxide was then added and the curve replotted. From these two curves the pKa values were found.

The corresponding pKa values at  $37^{\circ}\text{C}$ . are lower than those found  $25^{\circ}\text{C}$ . The relationship is:-

$$\log \frac{K_2}{K_1} = \frac{\Delta H}{4.576} \left( \frac{1}{T_1} - \frac{1}{T_2} \right)$$

Thus if the dissociation constant of a compound is known at two temperatures  $\Delta H$  can be calculated, and this may be used to find values of /



of pKa at other temperatures.

This was not considered necessary as it appeared that the approximate correction suggested by Albert (1960) was sufficiently accurate for our purposes. He noted that for weak bases a correction of -0.011 of a pH unit should be made for each °C. rise in temperature, and that for strong bases a correction of -0.022 was necessary.

The values found for the second pKa value of nicotine at two temperatures are:-

8.11 at 20°C. - Taylor (1951)  
and 8.01 at 25°C. - see results section,  
i.e. a fall of 0.02 units for each °C. rise in temperature.

The pKa at 37°C. was, in these experiments, calculated by subtracting 12 x 0.02 (i.e. 0.24) from the value at 25°C.

From the value of pKa it was possible to calculate the percentage ionised form present at physiological pH and temperature,

$$(\% \text{ ionised}) = \frac{100}{1 + 10^{(\text{pH} - \text{pKa})}}$$

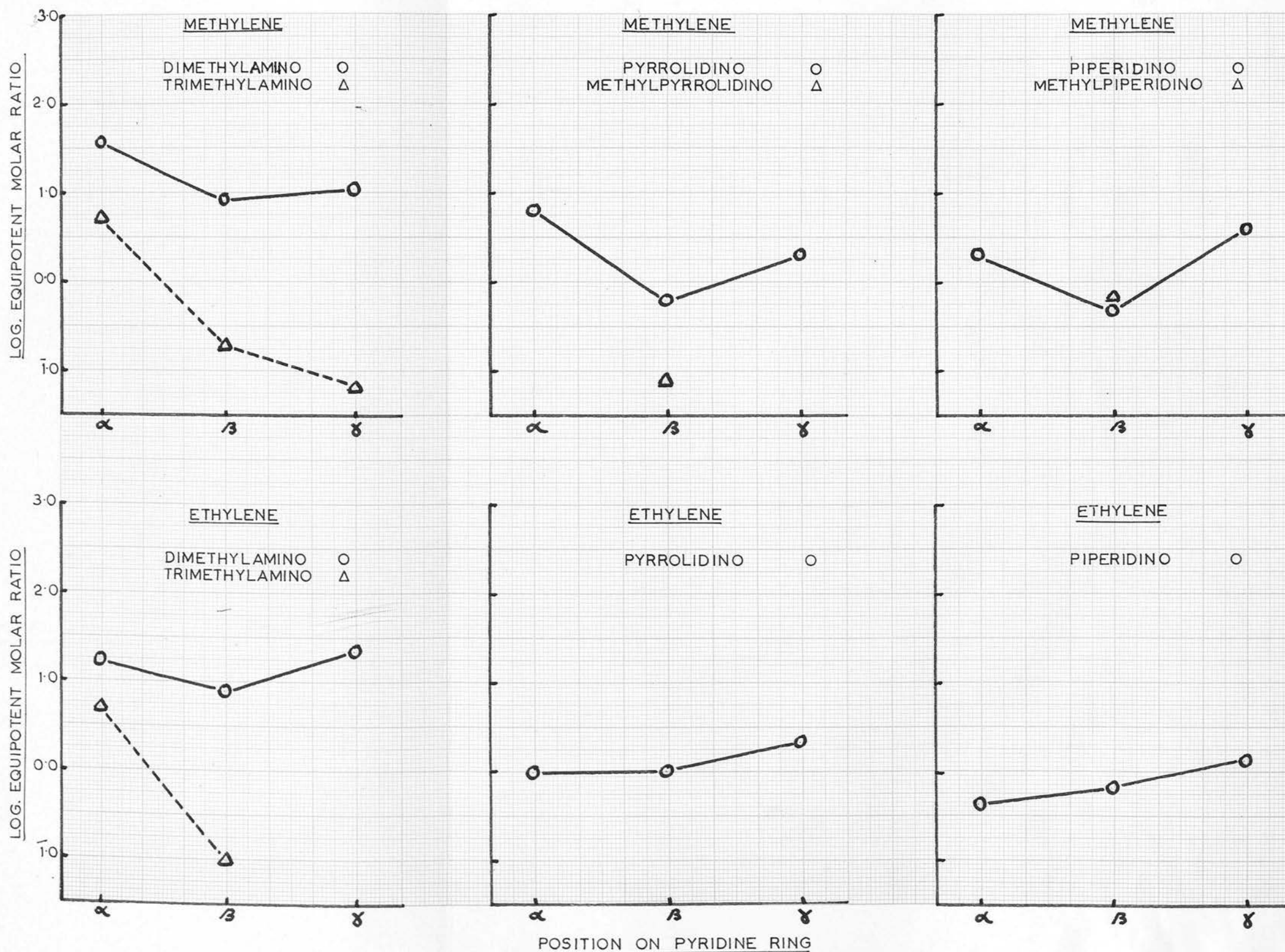


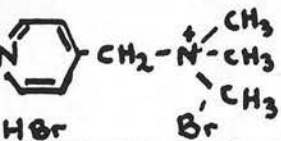
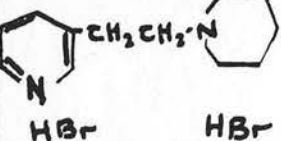
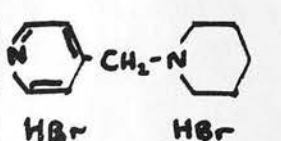
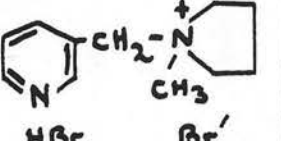
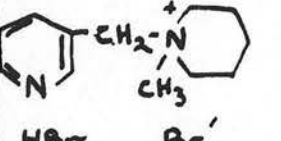
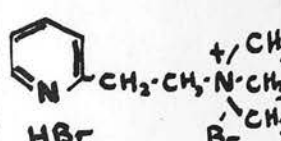
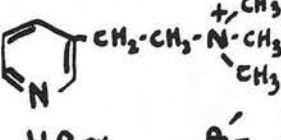
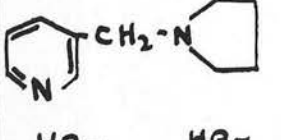
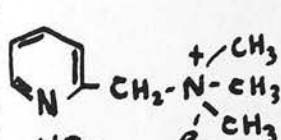
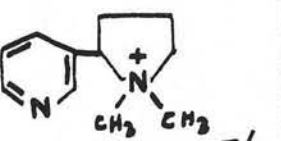
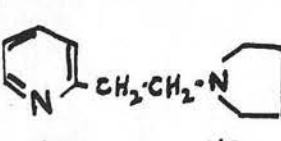
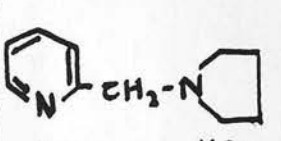
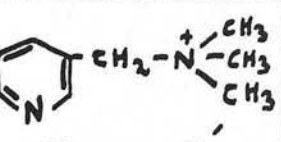
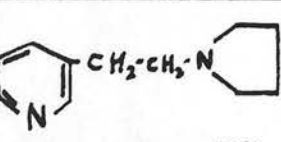
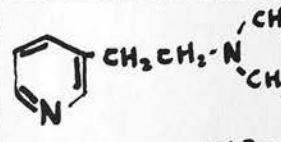
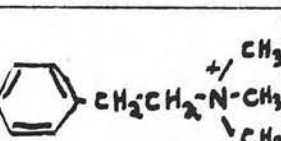
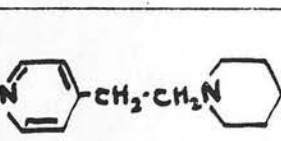
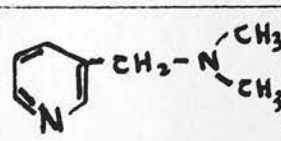
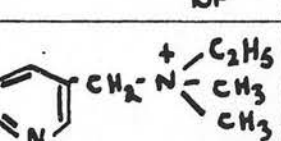
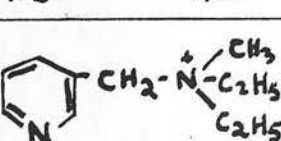
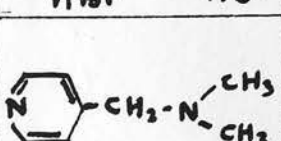
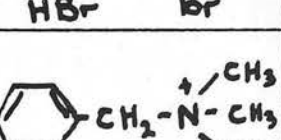
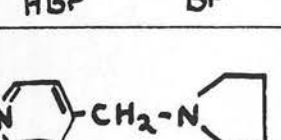
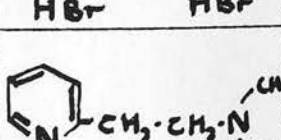
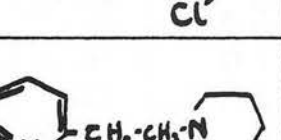
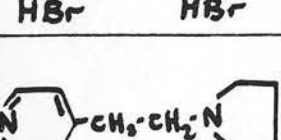
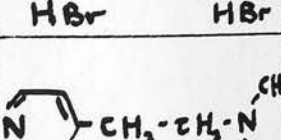
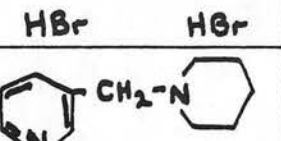
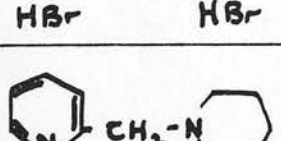
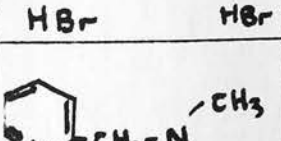
## EXPERIMENTAL RESULTS

GRAPH V

Log Equipotent Molar Ratio/  
Position on Pyridine Ring

- Rat Diaphragm -



COMPOUND	RATIO	EXP	COMPOUND	RATIO	EXP	COMPOUND	RATIO	EXP
 HBr	0.063 (16)	1(a)	 HBr	0.65 (1.5)	2(a)	 HBr	4.1	2(b)
 HBr	0.075 (13.3)	1(a)	 HBr	0.66 (1.5)	1(a)	 HBr	5.0	1(b)
 HBr	0.100 (10)	1(a)	 HBr	0.72 (1.4)	2(b)	 HBr	5.0	1(a)
 HBr	0.107 (9.3)	1(b)	 HBr	0.98 (1.0)	1(a) 1(b)	 HBr	6.7	1(a) 4(b)
 HBr	0.180 (5.5)	1(a)	 HBr	1.00	2(a)	 HBr	8.3	2(b)
 HBr	0.302 (3.3)	1(a)	 HBr	1.4	2(a)	 HBr	8.4	3(b)
 HBr	0.370 (2.7)	1(a)	 HBr	1.5	1(a)	 HBr	11.1	4(b)
 HBr	0.380 (2.7)	2(a)	 HBr	2.0	1(a) 1(b)	 HBr	16	3(a)
 HBr	0.430 (2.3)	2(a)	 HBr	2.1	2(a)	 HBr	22	3(a)
 HBr	0.500 (2.0)	2(a)	 HBr	2.3	2(a)	 HBr	37	1(a) 1(b)

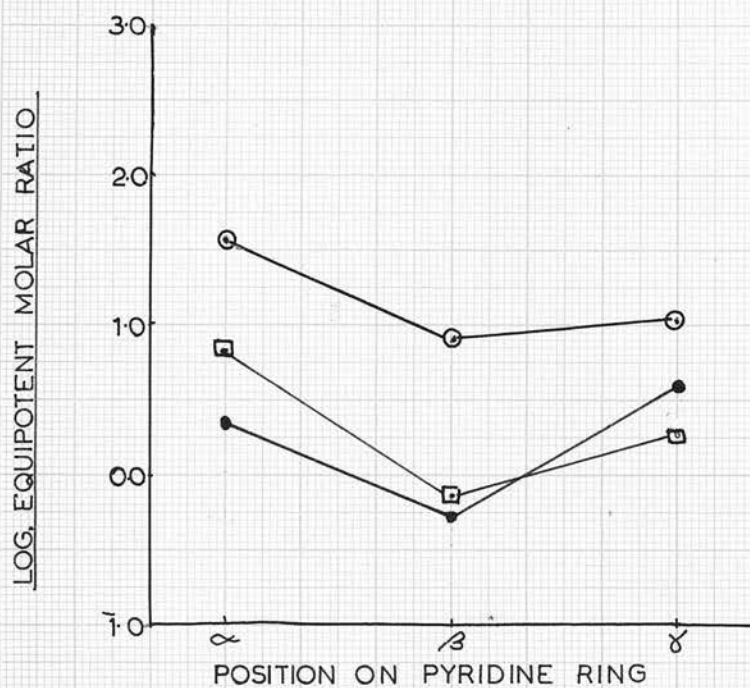
TABLE

RAT PHRENIC NERVE-DIAPHRAGM  
NEUROMUSCULAR BLOCKADE.

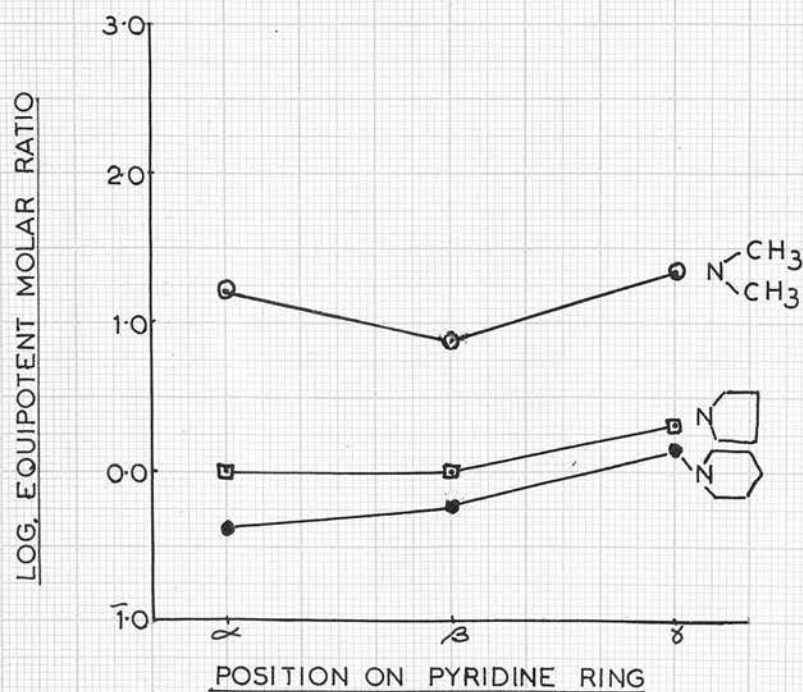
RATIO= EQUIPOTENT MOLAR RATIO  
EXP= NUMBER of EXPERIMENTS  
TYPE (a) GRAPHICAL  
(b) 4 POINT

RAT PHRENIC NERVE-DIAPHRAGM  
NEUROMUSCULAR BLOCKADE

METHYLENE



ETHYLENE





## RESULTS

### Activity at the Neuromuscular Junction:-

#### I The Rat Phrenic Nerve - Diaphragm Preparation:-

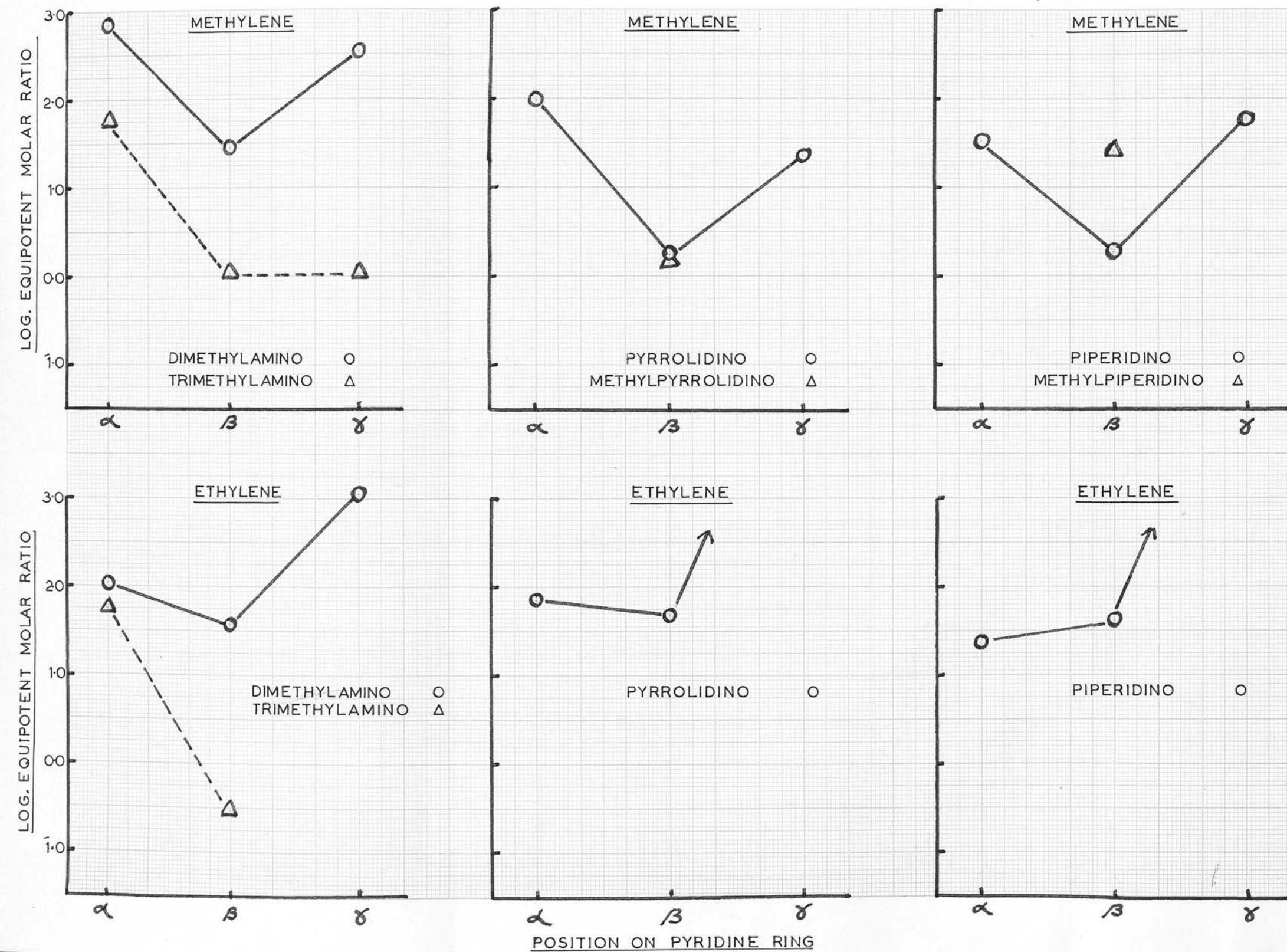
The neuromuscular blocking activity of the compounds tested on this preparation is summarised in Table 6 and Graphs V and VI. In the table the compounds are listed in order of activity.

The most active compound is  $\gamma$ -pyridyl methyl trimethylammonium bromide hydrobromide which is about 16 times as active as nicotine. Other compounds with high activity are  $\beta$ -pyridyl methyl methylpyrrolidinium bromide hydrobromide,  $\beta$ -pyridyl ethyl trimethylammonium bromide hydrobromide, and nicotine monomethiodide which are approximately 13, 10 and 9 times as active as nicotine respectively. On this preparation 14 out of 30 analogues of nicotine were more active than nicotine.

The quaternary compounds appear to be more active than the corresponding tertiary bases. Of the tertiary bases the  $\beta$  compounds appear to be more active than the corresponding  $\alpha$  and  $\gamma$  compounds although the  $\alpha$ -pyridyl ethyl piperidine and pyrrolidine dihydrobromides are exceptions. These compounds are 2.3 and 1.0 times as active as nicotine respectively.

Log Equipotent Molar Ratio/  
Position on Pyridine Ring

- Chick biventer cervicis -



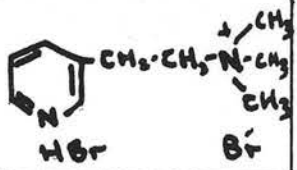
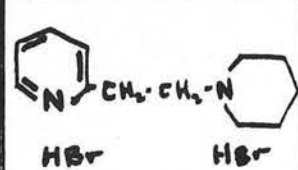
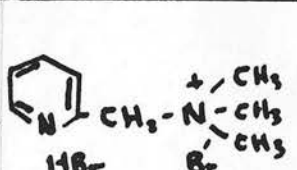
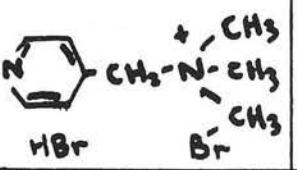
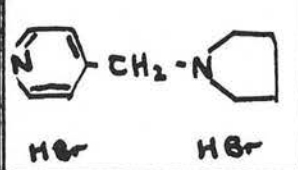
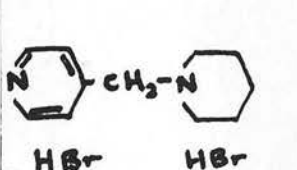
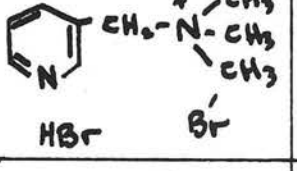
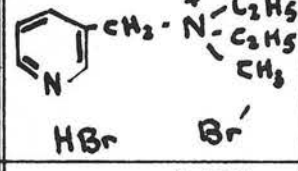
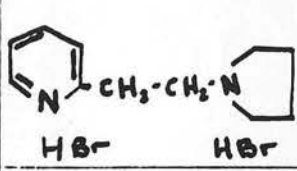
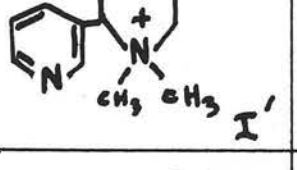
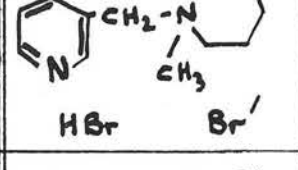
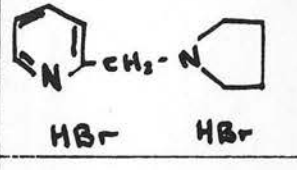
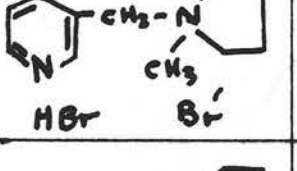
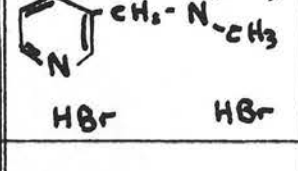
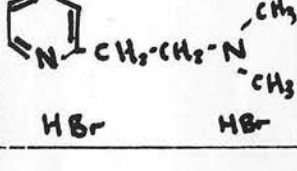
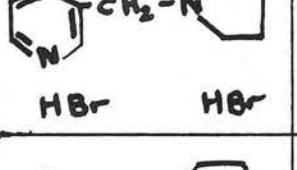
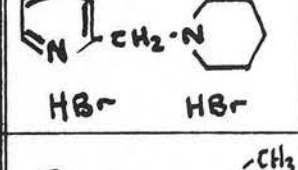
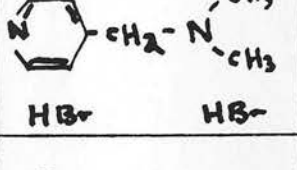
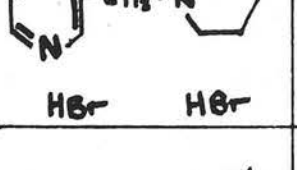
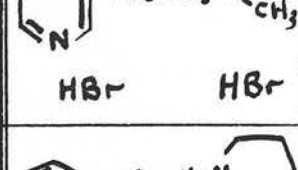
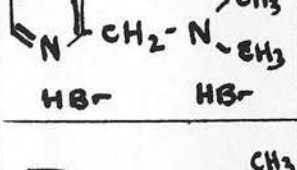
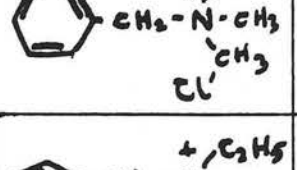
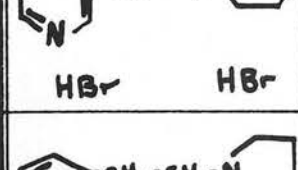
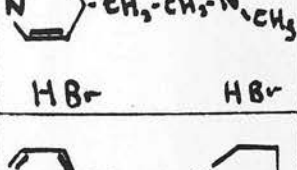
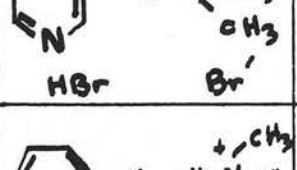
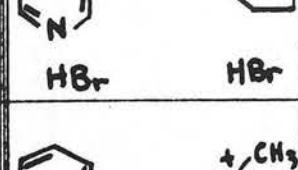
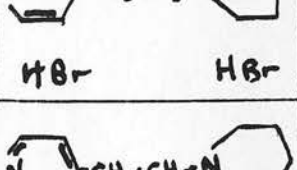

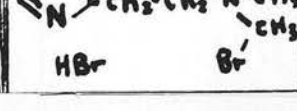
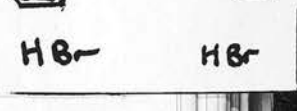


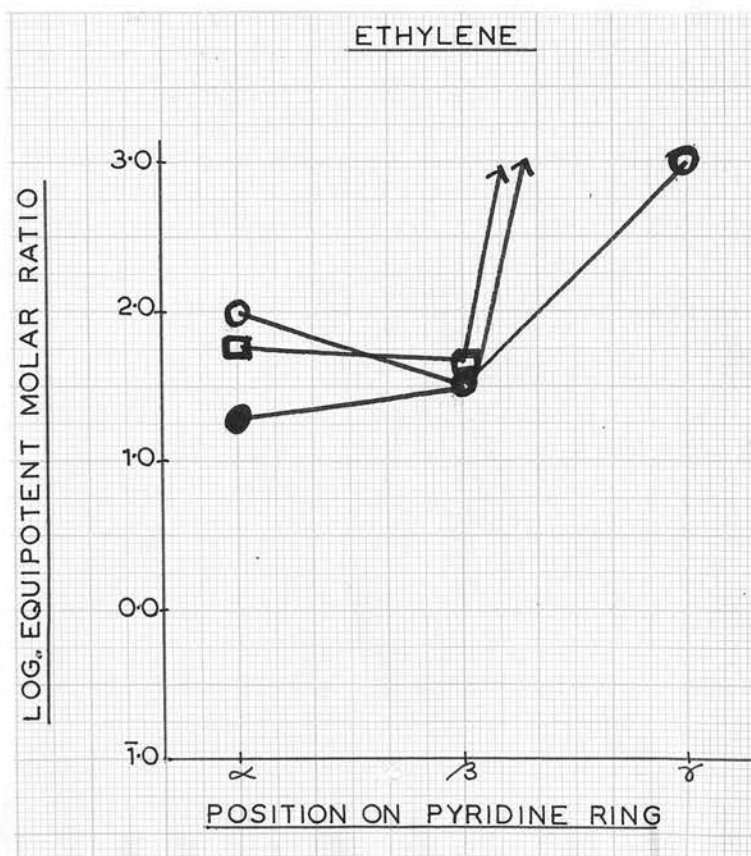
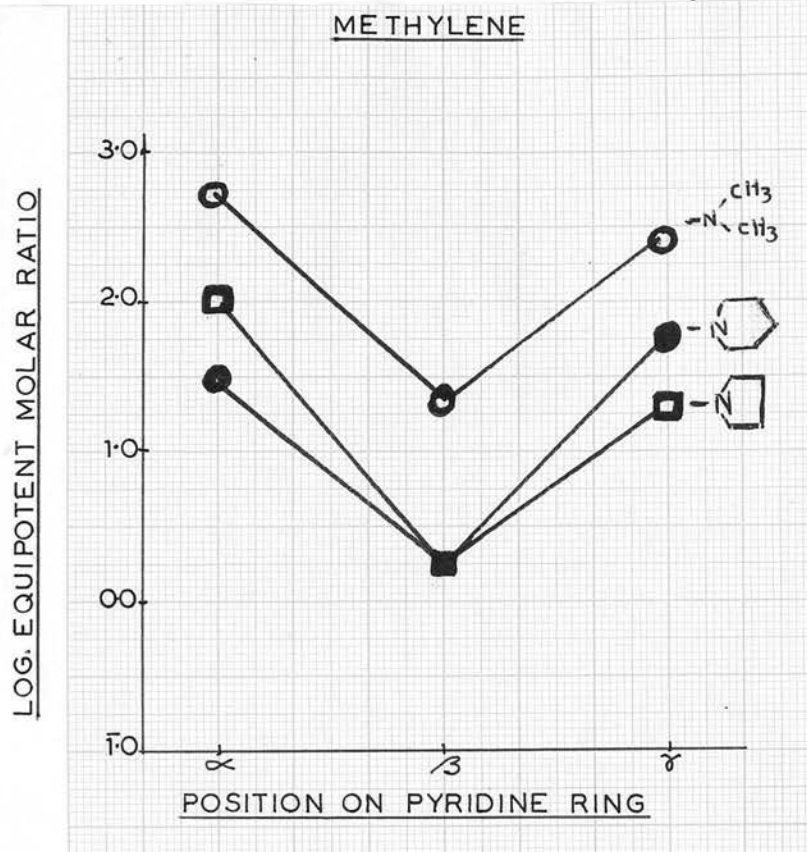
TABLE

CHICK BIVENTER CERVICIS  
CONTRACTURERATIO  $\equiv$  EQUIPOTENT MOLAR RATIOEXP  $\equiv$  NUMBER of EXPERIMENTS

TYPE (a) GRAPHICAL

(b) 4 POINT

COMPOUND	RATIO	EXP	COMPOUND	RATIO	EXP	COMPOUND	RATIO	EXP
	0.28	1(a)		22	1(a) 2(b)		>55	1(b)
	1.00	1(a)		22	2(b)		57	2(a)
	1.00	1(a)		25	1(a)		62	1(a) 1(b)
	1.2	2(a)		25	1(a)		91	2(b) 1(b)
	1.5	2(a)		28	2(a)		100	2(a)
	1.7	3(a) 1(b)		32	3(a)		317	2(a)
	1.8	4(a)		36	1(a) 1(b)		674	2(a)
	4.2	1(a)		40	2(a)		1086	3(a)
	4.6	1(a)		47	1(a) 1(b)		$\infty$	2(a)
	8.7	1(a)		55	1(a)		$\infty$	2(a)

CHICKEN BIVENTER CERVICIS  
CONTRACTURE

## II The Chick Biventer Cervicis Preparation:-

The activity of the compounds in producing contracture of the chick biventer cervicis preparation is summarised in Table 7 and Graphs VII and VIII. In Table 7 the compounds are listed in order of activity.

The only compound tested which is more active than nicotine (3.6 times) is  $\beta$ -pyridyl ethyl trimethylammonium bromide hydrobromide. Quaternisation of the dimethylamino side chain leads to an increase in the activity but quaternisation of heterocyclic substituents appears to have no effect on activity or may decrease activity, eg. nicotine monomethiodide is 0.8 times as active as nicotine and  $\beta$ -pyridyl methyl methylpiperidinium bromide hydrobromide is 0.07 times as active as its tertiary analogue.

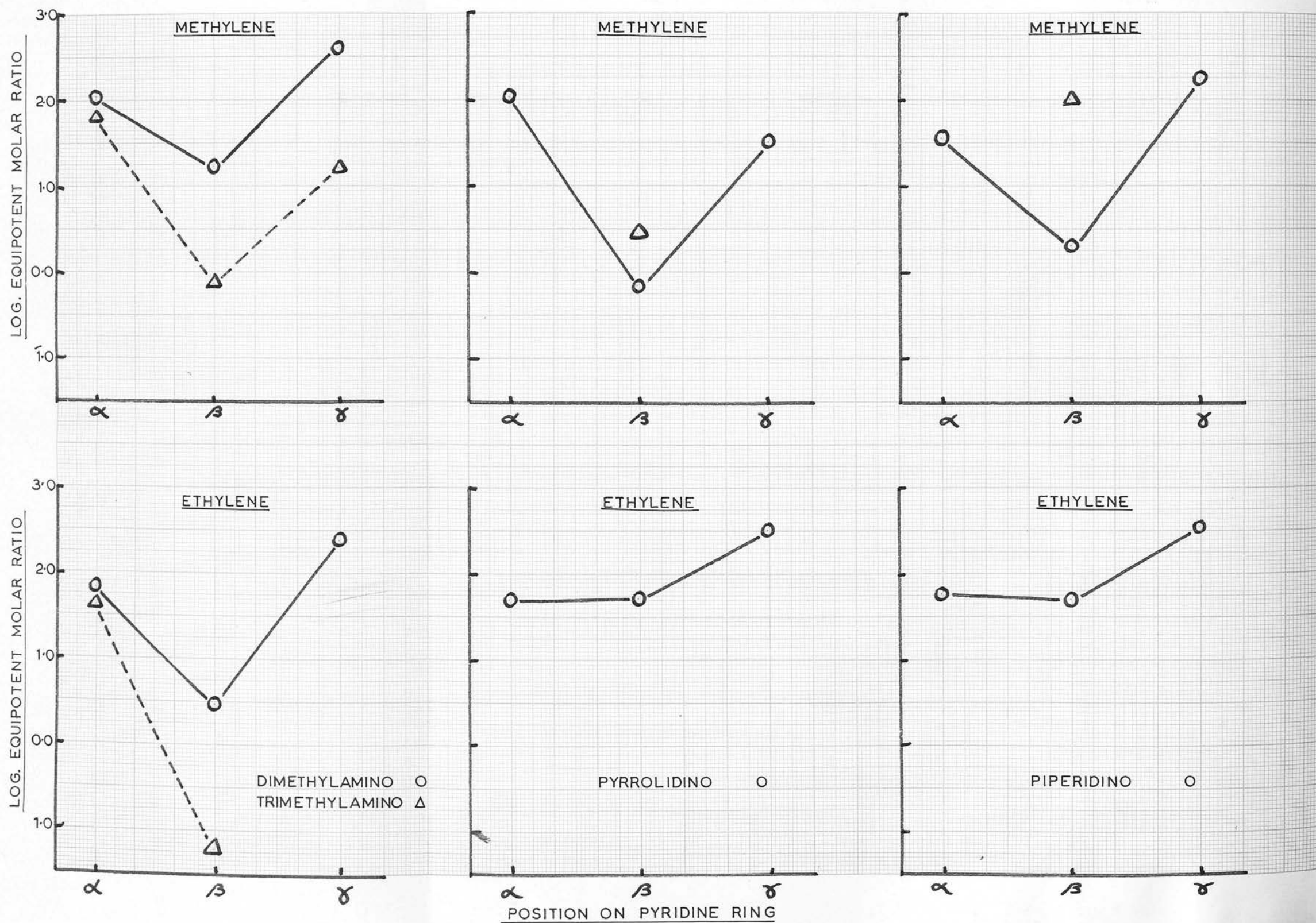
In the tertiary series the  $\beta$  compounds are more active than the corresponding  $\alpha$  and  $\gamma$  compounds with the exception of  $\alpha$ -pyridyl ethyl piperidine dihydrobromide.



GRAPH IX

Log Equipotent Molar Ratio/  
Position on Pyridine Ring

- Cat Superior Cervical Ganglion -



COMPOUND	RATIO	EXP	COMPOUND	RATIO	EXP	COMPOUND	RATIO	EXP
	0.06	2(b)		17	1(a) 1(b)		63	1(a)
	0.70	2(a) 2(b)		17	2(a)		67	2(a)
	0.73	1(a)		29	1(a)		100	1(a)
	2.0	1(a)		33	3(a) 1(b)		108	2(a)
	2.9	2(a)		35	2(a)		113	2(a)
	2.8	3(a)		41	2(a)		175	2(a)
	4.3	3(a) 1(b)		49	1(a)		237	2(a)
	9.3	1(a)		52	1(a)		330	2(a)
	14	1(a)		54	2(a)		359	3(a)
	16	1(a)		59	2(a)		400	1(a)

TABLE  
CAT SUPERIOR CERVICAL GANGLION  
BLOCKADE

RATIO = EQUIPOTENT MOLAR RATIO

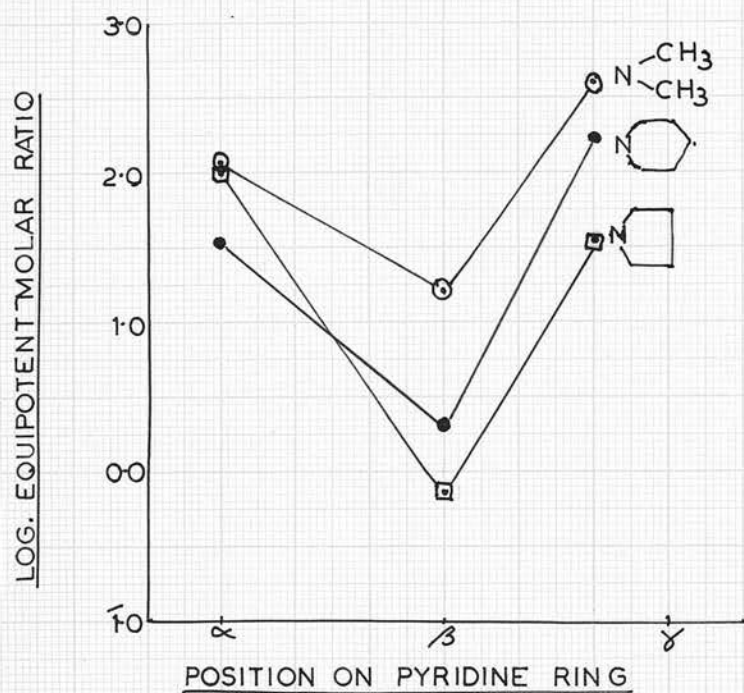
EXP = NUMBER OF EXPERIMENTS

TYPE (a) GRAPHICAL

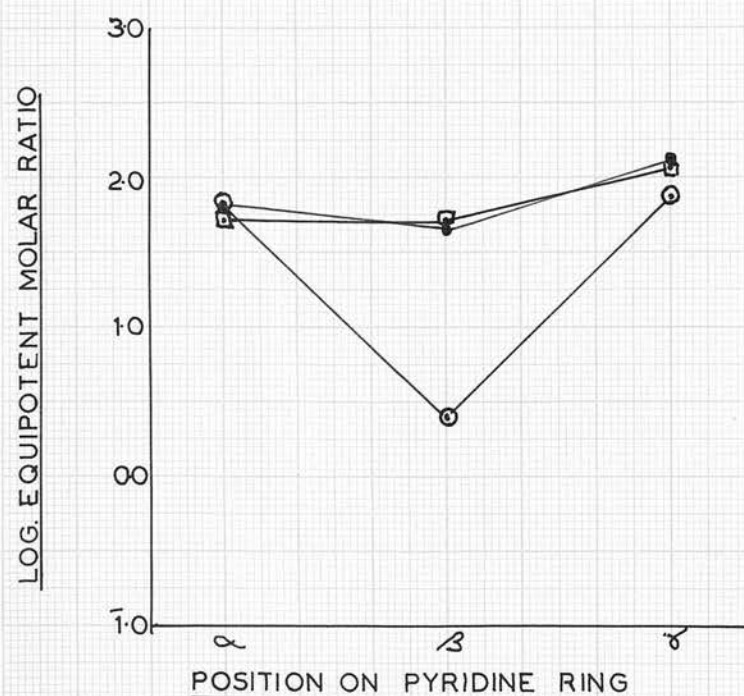
(b) 4 POINT

# CAT SUPERIOR CERVICAL GANGLION BLOCKADE

## METHYLENE



## ETHYLENE





### Activity at Ganglia

#### The Cat Superior Cervical Ganglion:-

(a) The blocking activity of the compounds on the cat superior cervical ganglion is summarised in Table 8 and Graphs IX and X. In Table 8 the compounds are listed in order of activity.














The most active compound is  $\beta$ -pyridyl ethyl trimethylammonium bromide hydrobromide which is 16 times as active as nicotine. The only other compounds which are more active than nicotine are  $\beta$ -pyridyl methyl pyrrolidine dihydrobromide and  $\beta$ -pyridyl methyl trimethylammonium bromide and hydrobromide which are both about 1.4 times as active as nicotine.

Quaternisation in the dimethylamino series increases activity whereas quaternisation of a heterocyclic side chain nitrogen always decreased activity. For example nicotine monomethiodide is 0.23 times as active as nicotine.

In both the tertiary and quaternary series the  $\beta$  compounds are more active than the corresponding  $\alpha$  and  $\gamma$  compounds.

Table 9

Stimulant Activity on Ganglion  
Observation on Nictitating Membrane

Compound	Exp.	Equipotent Molar Ratio	
		Stimulant	Blocking
 <chem>CN(C)CCc1ccccn1</chem>	1(b)	25	17
 <chem>CN(C)CCc1ccncc1</chem>	1(a)	400	400
 <chem>C1CCN(C1)CCc2ccccn2</chem>	1(a)	80	54
 <chem>C1CCN(C1)CCc2ccncc2</chem>	1(a)	33	52
 <chem>C1CCN(C1)CCc2ccccn2</chem>	1(a)	>800	330
 <chem>C1CCN(C1)CCc2ccccn2</chem>	1(a)	4.4	49
 <chem>CN(C)CCc1ccccn1</chem>	1(a)	2.4	2.9
 <chem>C[N+](C)(C)CCc1ccccn1</chem>	1(a)	0.5	0.73
 <chem>CC[N+](CC)(CC)CCc1ccccn1</chem>	1(a)	1.3	9.3
 <chem>C[N+]1CCCC1CCc2ccccn2</chem>	1(b)	3.5	4.3
 <chem>C[N+]1(C)CCCC1OCCc2ccccn2</chem>	1(b)	0.18	0.49
 <chem>C[N+]1(C)CCCC1OCCc2ccccn2</chem>	1(a)	inactive	3.0
 <chem>C[N+]1(C)CCCC1CCc2ccccn2</chem>	1(a)	inactive	16

(a) Graphical Assay  
(b) k-point Assay

The Cat Superior Cervical Ganglion

(b) The stimulant activity of a few of the compounds is summarised in Table 9. In all of the other compounds tested only a qualitative estimate of stimulant activity was made.

All the compounds tested (apart from two) produced, in doses which caused ganglion block, an initial stimulation of the ganglion as shown by a rapid contraction of the nictitating membrane. The only two compounds which failed to stimulate were  $\beta$ -pyridyl methyl methyldiethylammonium bromide hydrobromide and homocholine phenyl ether.

Insufficient quantitative estimates of stimulant activities were made to warrant any conclusions being drawn from these results although it is interesting to note that for the quaternary compounds tested the stimulant activity compared to nicotine was greater than the blocking activity found.

Table 10 Anticholinesterase Activity: Inhibitor Constant  $K_i$

Tertiary Compounds

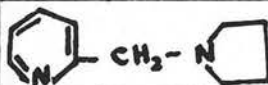
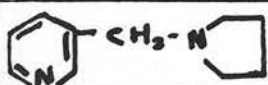
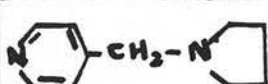
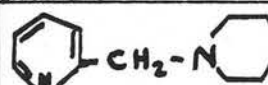
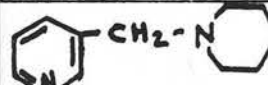
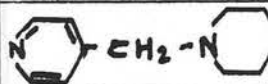
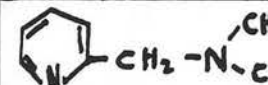
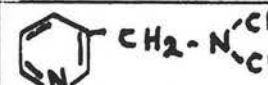
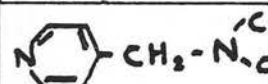
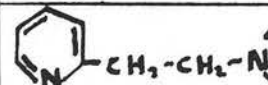
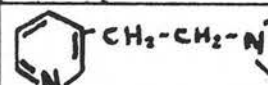
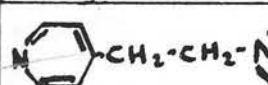
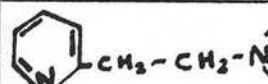
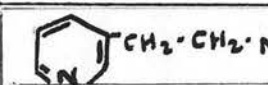
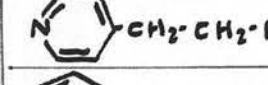
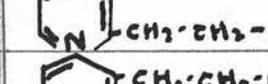
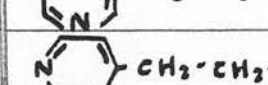
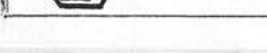

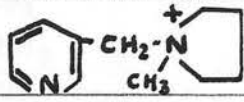
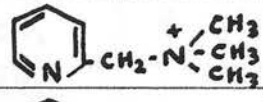
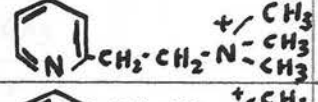
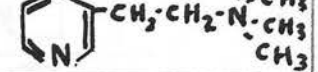
Compound	Exps	Conc <sup>n</sup> . 50% inhib. $\times 10^{-3}$	$-\log K_i$ ( $pI_{50} + 0.59$ )	Inhibitor Constant $K_i$	
Nicotine	3	2.60	3.18	$6.61 \times 10^{-4}$	
	3	1.05	3.57	$2.69 \times 10^{-4}$	(3)
	3	1.99	3.29	$5.13 \times 10^{-4}$	(5)
	3	8.96	2.64	$2.29 \times 10^{-3}$	
	3	2.42	3.21	$6.17 \times 10^{-4}$	
	3	3.51	3.04	$9.12 \times 10^{-4}$	
	3	5.38	3.26	$5.50 \times 10^{-4}$	
	2	2.31	3.23	$5.89 \times 10^{-4}$	
	2	8.99	2.64	$2.29 \times 10^{-3}$	
	2	103	1.58	$2.63 \times 10^{-2}$	
	2	0.257	4.18	$6.61 \times 10^{-5}$	(2)
	2	94.6	1.61	$2.46 \times 10^{-2}$	
	2	5.53	2.85	$1.41 \times 10^{-3}$	
	3	1.42	3.44	$3.63 \times 10^{-4}$	(4)
	3	2.37	3.22	$6.03 \times 10^{-4}$	
	3	4.19	2.97	$1.07 \times 10^{-3}$	
	2	0.188	4.32	$4.79 \times 10^{-5}$	(1)
	2	4.55	2.93	$1.18 \times 10^{-3}$	
	2	10.2	2.58	$2.63 \times 10^{-3}$	

Table 11

Anticholinesterase Activity: Inhibitor Constant  $K_i$   
Quaternary Compounds

Compound	Exps	Conc <sup>n</sup> 50% inhib. $\times 10^{-3}$	$-\log K_i$ ( $pI_{50} + 0.59$ )	Inhibitor Constant $K_i$
	2	2.5	3.19	$6.5 \times 10^{-4}$
	2	2.7	3.16	$7.0 \times 10^{-4}$
	2	150	1.41	$3.9 \times 10^{-2}$
	2	6.0	2.81	$1.5 \times 10^{-3}$
	1	300	1.11	$7.8 \times 10^{-2}$

Activity on Cholinesterase: -

The anticholinesterase activity of the compounds on dog caudate nucleus is summarised in Tables 10 and 11. The right hand column in the tables gives an estimate of the Inhibitor Constants ( $K_i$ ) calculated by the method described above.

In general the  $\alpha$  compounds are more active than the corresponding  $\beta$  and  $\gamma$  compounds. The most active compound is  $\alpha$ -pyridyl ethyl dimethylamine dihydrobromide. The five most active compounds are numbered at the extreme right of the table in order of activity. In general the ethylene-linked compounds are more active than the corresponding methylene-linked compounds, (especially in the  $\alpha$  series).

The five quaternary compounds tested are less active than the corresponding tertiary compounds.



Table 12

pKa Values and % Ionisation of Tertiary Compounds

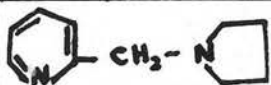
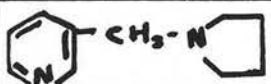
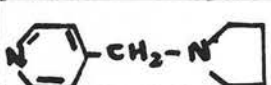
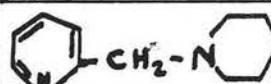
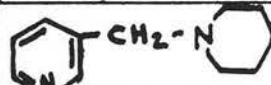
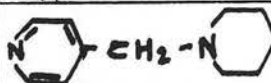
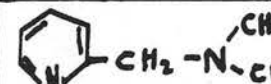
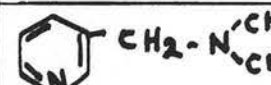
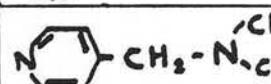
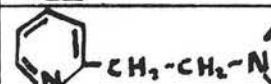
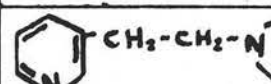
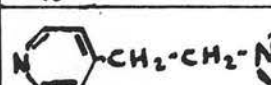
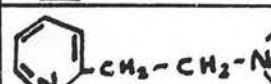
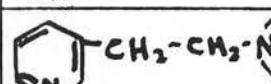
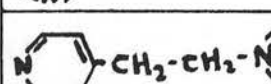
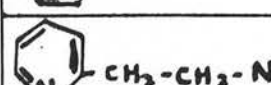
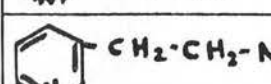
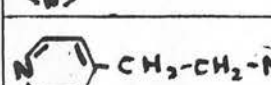
Compound	pKa Pyridine Nitrogen 25°C	pKa Side- chain Nitrogen 25°C	pKa Side- chain Nitrogen 37°C	% Ionised Form at 37°C and pH 7.38
Nicotine	3.10	8.01	7.77	71.5
	2.60	8.60	8.36	90.5
	3.20	8.40	8.16	85.7
	3.44	8.20	7.96	79.2
	2.67	8.55	8.31	89.4
	3.22	8.34	8.10	84.0
	3.96	7.92	7.68	66.7
	2.64	8.16	7.92	77.7
	3.23	8.04	7.80	72.4
	3.45	7.70	7.46	54.6
	3.66	9.43	9.19	98.4
	4.34	9.32	9.08	98.0
	4.71	9.31	9.07	98.0
	3.65	9.33	9.09	98.1
	4.31	8.85	8.61	94.4
	4.74	9.10	8.86	96.8
	3.52	8.79	8.55	93.6
	4.36	8.90	8.66	95.0
	4.72	8.74	8.50	93.0

Table 13

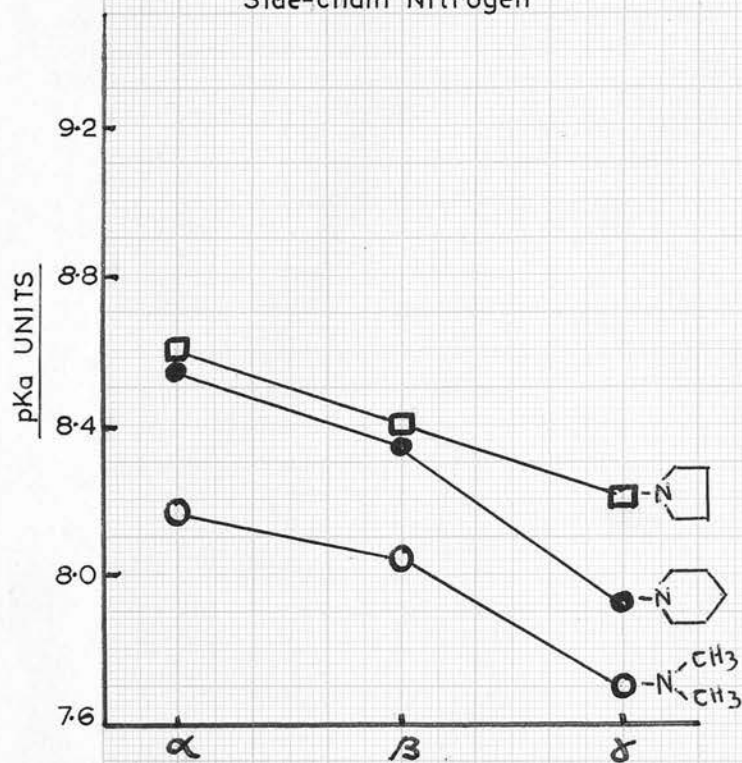
pKa Values for Pyridine Nitrogen  
of Quaternary Compounds

Compound	pKa 25°C	Compound	pKa 25°C
	3.15		3.13
	2.69		3.54
	3.10		4.35
	3.24		3.12
	3.13		3.11

GRAPH XI pKa's of SUBSTITUTED PYRIDINE COMPOUNDS

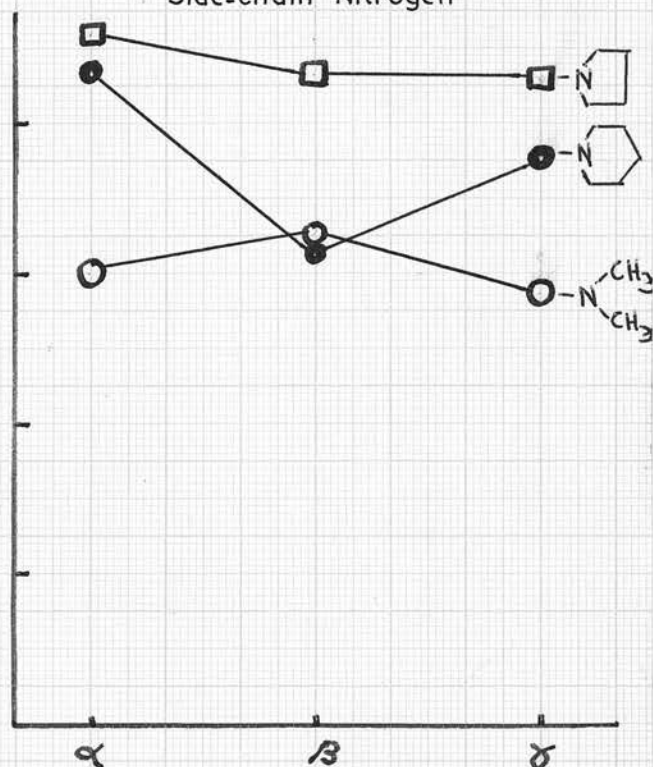
METHYLENE SERIES

Side-chain Nitrogen

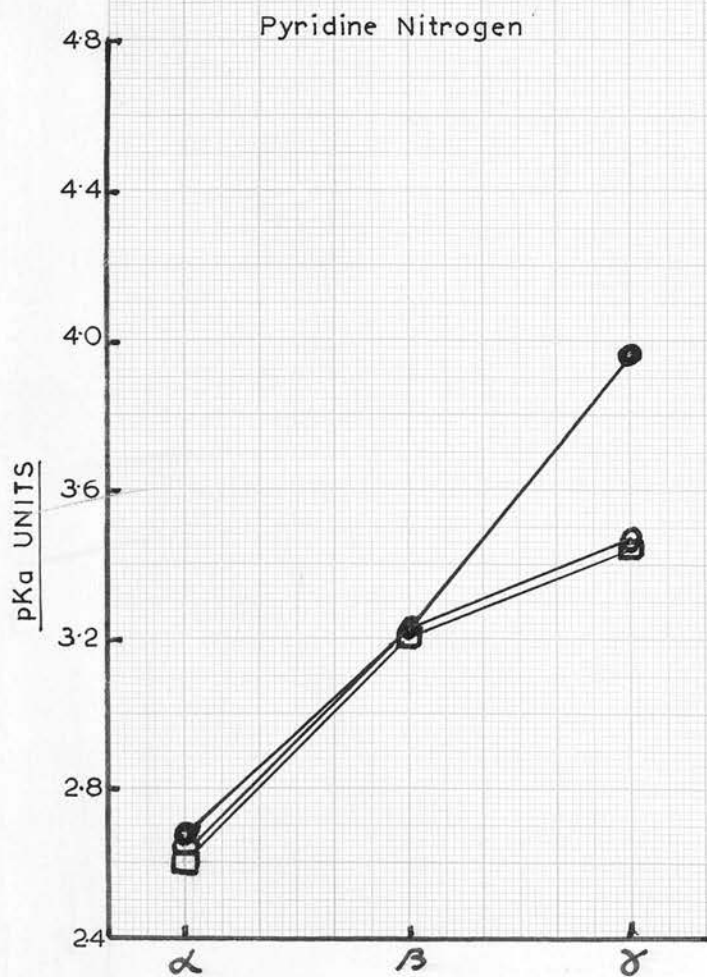


ETHYLENE SERIES

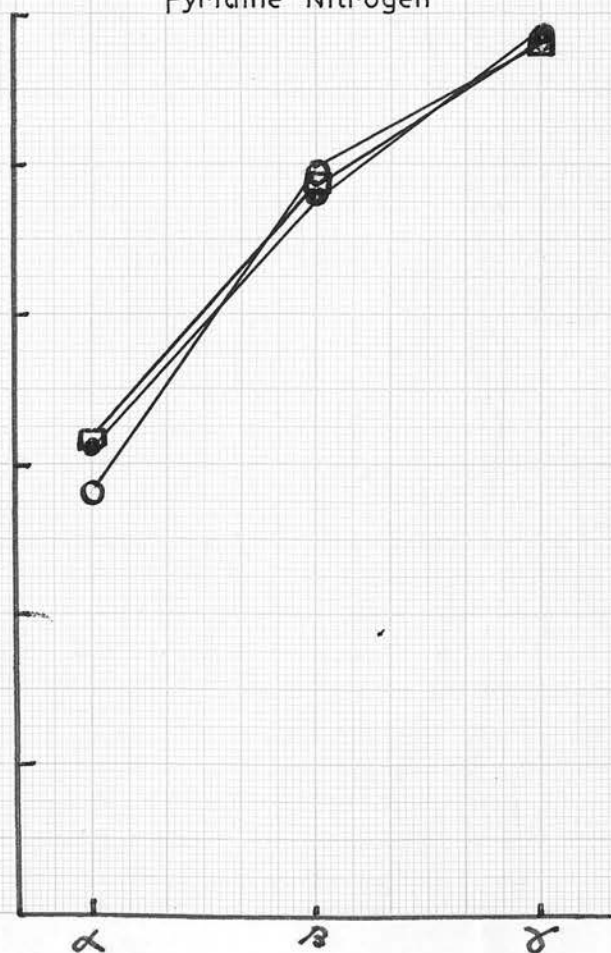
Side-chain Nitrogen



Pyridine Nitrogen



Pyridine Nitrogen



POSITION ON PYRIDINE RING

POSITION ON PYRIDINE RING

### Dissociation Constants

The dissociation constants of the compounds expressed as the pKa's at 25°C are summarised in Tables 12 and 13 and Graph XI.

The pKa value of the pyridine nitrogen increases as the substituent is moved from the  $\alpha$  to the  $\beta$  to the  $\gamma$  position in both tertiary and quaternary compounds of the methylene and ethylene series. These results suggest that the presence of the charge on the side chain nitrogen increases the dissociation of the proton from the pyridine nitrogen and hence the  $\alpha$  compounds are weaker bases than the  $\beta$  compounds which are weaker than the  $\gamma$  compounds. The increase in base strength as the chain length separating the side chain nitrogen from the pyridine ring is increased from methylene to ethylene gives further support to the above hypothesis.

In the ethylene series there is no regular pattern observed in the pKa values of the side chain nitrogen. In the methylene series, however, with the three nitrogen substituents tested there is a decrease in the pKa value as the side chain is moved from the  $\alpha$  to the  $\beta$  to the  $\gamma$  position. This increase in base strength /

strength as the side chain nitrogen approaches the pyridine nitrogen may be due to the presence of a negative charge on the pyridine nitrogen. (Longuet-Higgins and Coulson 1947). Such a negative charge may attract a proton and assist its binding to the side chain nitrogen if this is close (as in the  $\alpha$  position) and thus may cause an increase in the base strength of the side chain nitrogen.

## DISCUSSION

### I Significance of the Results:-

#### Type of Action on the Rat Diaphragm:

Activity has been studied at two types of site, the neuromuscular junction and the ganglion.

In the rat diaphragm test a block of conduction could be brought about (setting aside an interference with the release of transmitter) by an action like curare or by an action like decamethonium. From the account in the introduction it seems reasonable to regard the action of decamethonium as being one of desensitisation associated with a brief depolarisation. A measure of ability to depolarise should be provided fairly reliably by the contracture produced in the chick biventer test (allowance being made for species differences). This may give some indication of the ability to produce a desensitisation block and, if this is true, differences between activity in the chick biventer test and in the rat diaphragm test might be taken to indicate differences in the way neuromuscular block is produced. /



produced. For example 8 -pyridyl ethyl piperidine and pyrrolidine dihydrobromides are virtually inactive in the chick biventer test and yet are comparable in activity with nicotine in the rat diaphragm test: this suggests a curare-like action on the rat diaphragm.

#### Type of Action on the Cat Ganglion:

In ganglia (again setting aside interference with the release of transmitter) two types of action are possible, a block like hexamethonium (apparently competitive) and a block like acetylcholine or nicotine (apparently one of desensitisation). Some indication of the ability to desensitise might be provided by the ability of the drug to cause contracture of the unstimulated nictitating membrane. This was not studied systematically but the information available (Table 9) suggests that most compounds do this and are probably acting like nicotine. Although it is very doubtful whether the results with the chick biventer can be expected to indicate ability to desensitise the ganglion, there is in fact quite good agreement between the two preparations (Tables 17 and 18).

#### Possibility of an Indirect Action:

##### Anticholinesterase Activity:

In all these tests the possibility of an indirect action by blocking cholinesterase must be /

Table 14

Comparison of Effective Concentrations  
Tertiary Compounds

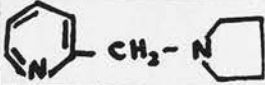
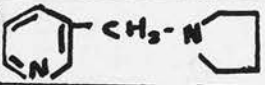
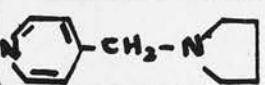
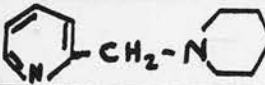
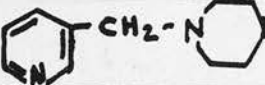
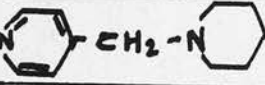
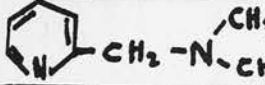
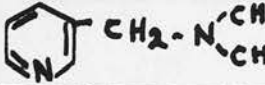
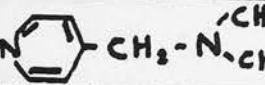
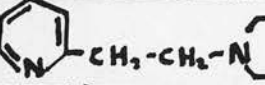
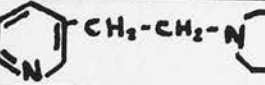
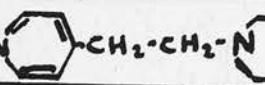
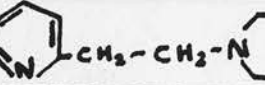
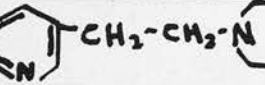
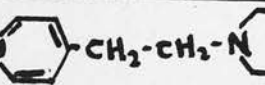
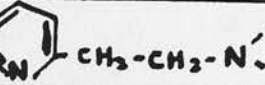
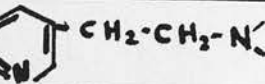
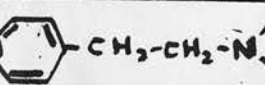
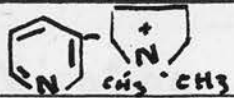
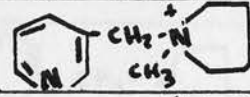
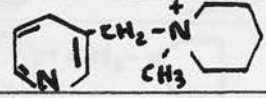
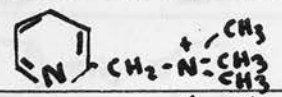
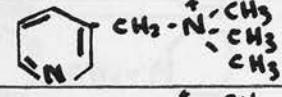
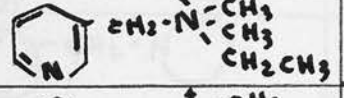
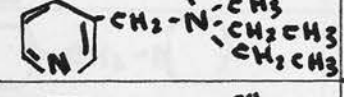
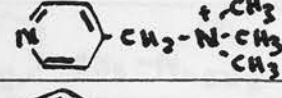
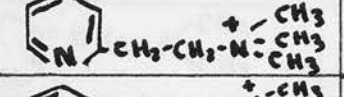
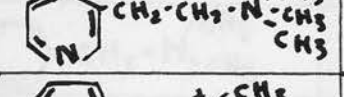
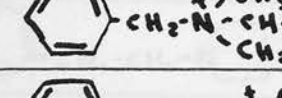
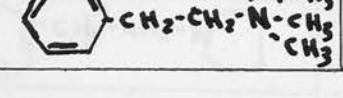
Compound	Rat -logEC <sub>50</sub>	Chick -logEC <sub>50</sub>	Cat -logED <sub>50</sub>	Cholin- esterase -log Ki
Nicotine	3.69	5.60	2.81	3.18
	2.88	3.65	0.78	3.57
	3.83	5.37	2.97	3.29
	3.39	5.26	1.29	2.64
	3.33	4.10	1.27	3.21
	3.99	5.35	2.51	3.04
	3.08	3.85	0.57	3.26
	2.12	2.78	0.76	3.23
	2.77	4.16	1.60	2.64
	2.65	3.10	0.21	1.58
	3.70	3.81	1.08	4.18
	3.69	3.94	1.10	1.61
	3.37	< 2.0	0.30	2.85
	4.06	4.26	1.04	3.44
	3.88	4.07	1.12	3.22
	3.54	< 1.83	0.26	2.97
	2.49	3.60	0.99	4.32
	2.77	4.05	2.35	2.93
	2.35	2.57	0.44	2.58

Table 15

Comparison of Effective Concentrations  
Quaternary Compounds

Compound	Rat -logEC <sub>50</sub>	Chick -logEC <sub>50</sub>	Cat -logED <sub>50</sub>	Cholin- esterase -log Ki
	4.66	5.54	2.18	3.19
	4.82	5.43	2.37	3.16
	3.87	4.21	0.81	-
	2.99	3.86	1.01	1.41
	4.43	5.60	2.95	-
	4.12	4.94	1.84	-
	3.51	4.21	1.62	-
	4.89	5.60	1.58	-
	2.99	3.86	1.20	2.81
	4.70	6.15	4.03	1.11
	4.11	4.98	1.35	-
	4.21	4.66	1.67	-

be considered. A comparison is made in Tables 14 and 15 between the concentrations of the compounds effective on the three preparations and cholinesterase from dog caudate nucleus. It appears that anticholinesterase activity is really only likely to affect the pharmacological response in the rat diaphragm. None of the quaternary compounds tested is likely to block cholinesterase to a significant degree in the concentrations used in the pharmacological tests.

The results in the ganglion experiments are more difficult to analyse because the effective concentration at the ganglion is unknown. As will be seen in Table 14 in these experiments the 0.1 ml. of a  $10^{-2.81}$  M solution of nicotine produced roughly 50% block. This can hardly be the concentration at the receptors because, if it were, a considerable block of cholinesterase should result and experiments do not support the idea that the ganglion blocking action of nicotine is accompanied by any appreciable block of cholinesterase. With the  $\alpha$  substituted pyridyl compounds, however, the figures for the injected concentration and effective anti-cholinesterase concentration differ by almost 1000 /

# GRAPH XII

## GRAPH

COMPARISON BETWEEN EFFECTIVE CONCENTRATION in CAT GANGLION  
and ANTICHOLINESTERASE CONC.  $(-\text{LOG.EC}_{50}) - (-\text{LOG.Ki})$  / NUMBER of  
COMPOUNDS

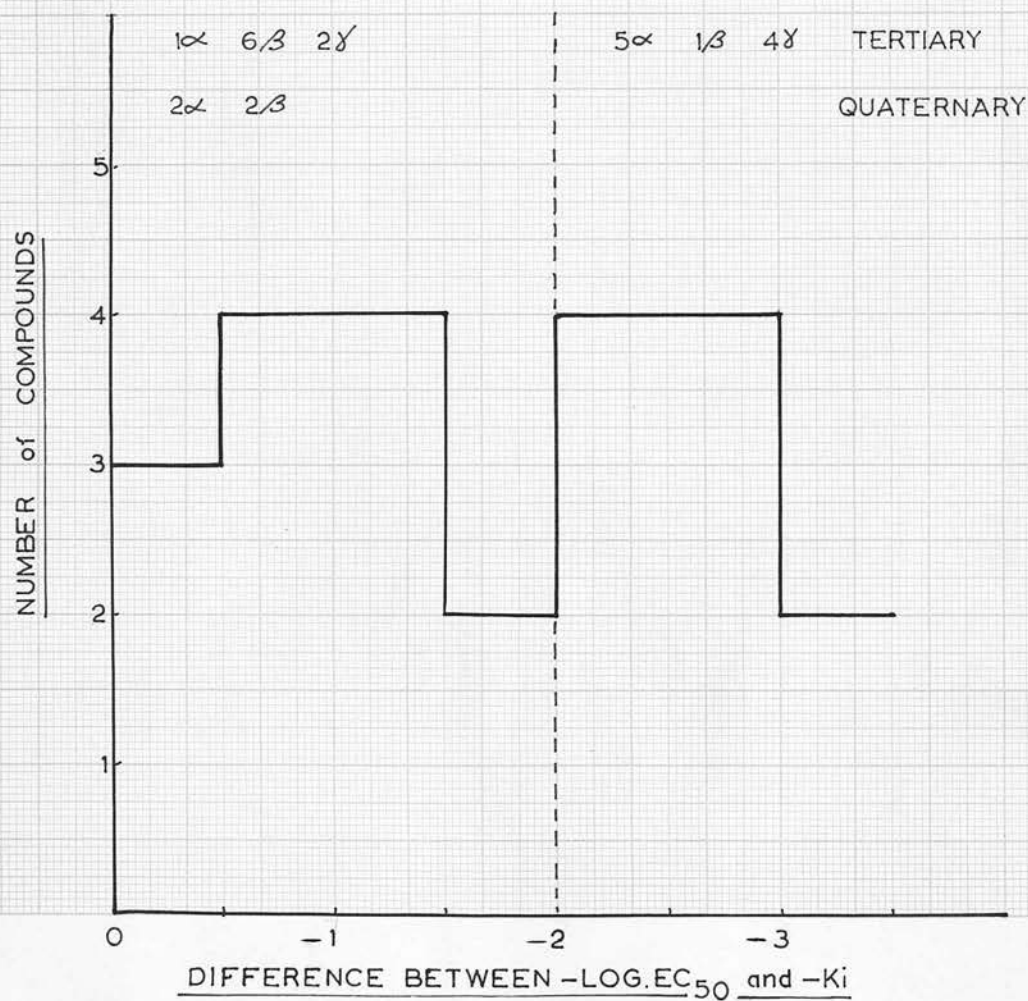




Table 16

Differences between:  $-\log EC_{50}$  and  $-\log Ki$ 

	Rat	Chick	Cat
Nicotine	+ 0.88	+ 2.42	- 0.37
	- 0.69	+ 0.08	- 2.79
	+ 0.54	+ 2.08	- 0.32
	+ 0.75	+ 2.62	- 1.35
	+ 0.12	+ 0.89	- 1.94
	+ 0.95	+ 2.31	- 0.53
	- 0.18	+ 0.59	- 2.69
	- 1.11	- 0.45	- 2.47
	+ 0.13	+ 1.52	- 1.04
	+ 1.07	+ 1.52	- 1.37
	- 0.48	- 0.37	- 3.10
	+ 2.08	+ 2.33	- 0.51
	+ 0.52	- 0.85	- 2.55
	+ 0.62	+ 0.82	- 2.40
	+ 0.66	+ 0.75	- 2.10
	+ 0.57	- 1.14	- 2.71
	- 1.83	- 0.72	- 3.33
	- 0.16	+ 1.12	- 0.58
	- 0.13	- 0.01	- 2.14
	+ 1.47	+ 2.35	- 1.01
	+ 1.66	+ 2.27	- 0.79
	+ 1.58	+ 2.45	- 0.40
	+ 0.18	+ 1.05	- 1.61
	+ 2.59	+ 5.04	+ 2.92



1000 and the type of response (long duration) suggests that these, at least, are affecting cholinesterase in the experiments. If the distribution of the difference between  $-\log K_i$  and  $-\log C_{50}$  is plotted (Graph XII) it will be seen that 5  $\alpha$  compounds, 4  $\gamma$  compounds, and 1  $\beta$  compound have values between -2.0 and -3.5 and 6  $\beta$  compounds, 1  $\alpha$  compound and 2  $\gamma$  compounds have values between -1.99 and -0.00. The quaternary compounds for which the figures are available (2  $\beta$  compounds and 2  $\alpha$  compounds) all have values between -1.61 and -0.40. (Table 16).

The compounds in which the difference lies between -2.0 and -3.5 appear to be either the  $\gamma$  tertiary compounds which are only feebly active and so must be given in a higher concentration, or the  $\alpha$  compounds whose anticholinesterase activity is greater than that of the  $\beta$  and  $\gamma$  compounds (Table 10). It might be supposed, therefore, that the dilution factor is of the order of 100 but this is clearly only a guess.

Active /

Active Species: Ion or Salt:

Before relationships between structure and activity can be discussed it is important to consider whether it is the ion or the base (or both) which is active. This could be settled by studying the effect of pH on activity. This has not been done (as yet) but some indication of which is the active species is given by considering the degree of ionisation of the various compounds at the pH of the test (7.38). All the compounds are mostly in the form of the univalent ion in which the proton is attached to the side chain amino group. Even the compound with the least basic side chain amino group (  $\delta$  -pyridyl methyl dimethylamine dihydrobromide) is over 50% ionised and most of the compounds are round about 90% ionised (Table 12, Graph XI). In contrast the pyridine nitrogen atom, even in the most strongly basic compound (  $\delta$  -pyridyl ethyl piperidine dihydrobromide) is at most 0.25% ionised. This coupled with the activity of the quaternary compounds strongly suggests that it is the univalent cation which is active. If this is /

Table 17

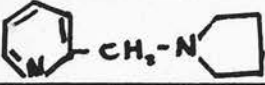
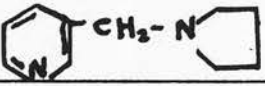
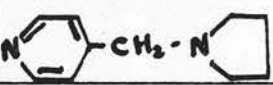
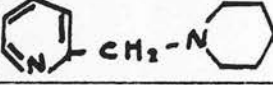
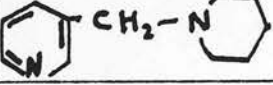
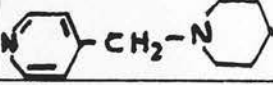
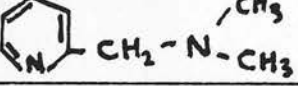
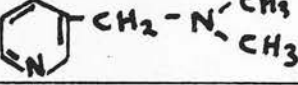
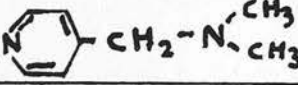
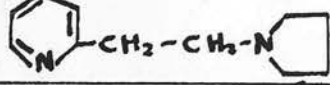
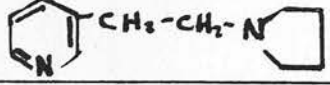
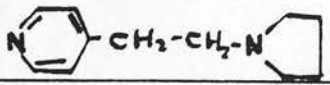
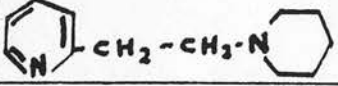
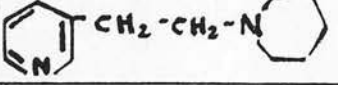
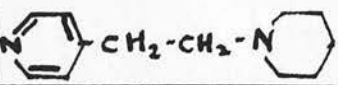
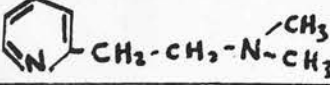
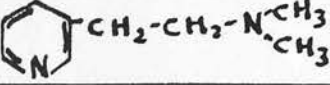
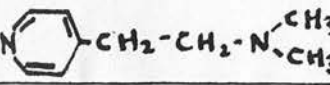
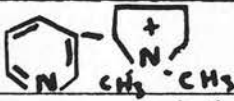
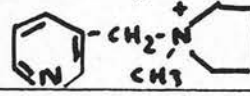
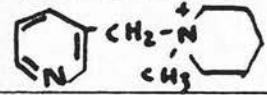
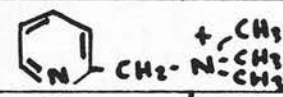
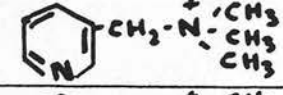
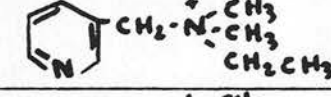
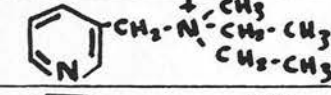
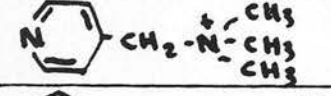
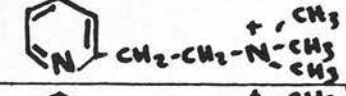
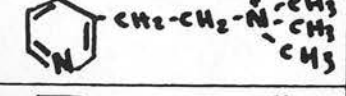
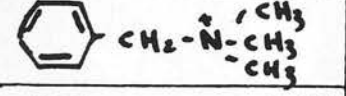
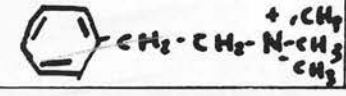
Compound	Equipotent Molar Ratio					
	Rat Diaphragm		Chick biventer		Cat Ganglion	
	Total Salt	ion	Total Salt	ion	Total Salt	ion
Nicotine	1.0	1.0	1.0	1.0	1.0	1.0
	6.7	8.5	91	115	108	137
	0.72	0.86	1.7	2.04	0.7	0.84
	2.0	2.22	22	24.4	33	37
	2.3	2.9	32	40	35	44
	0.5	0.59	1.8	2.1	2.0	2.35
	4.1	3.8	57	53	175	163
	37	40	674	732	113	123
	8.4	8.5	28	28.3	17	17.2
	11	8.4	317	242	400	306
	0.98	1.35	62	85	54	74
	1.0	1.37	47	64	52	71
	2.1	2.88	>4000	>4000	330	452
	0.43	0.59	22	30	59	81
	0.65	0.86	40	53	49	65
	1.4	1.9	>6000	>6000	359	486
	16	21	100	131	67	88
	8.3	11	36	48	2.9	3.9
	22	29	1086	1413	237	308

Table 18

## Collected Results : Quaternary Compounds

Compound	Equipotent Molar Ratio					
	Rat Diaphragm		Chick biventer		Cat Ganglion	
	cf Nicotine salt	cf Nicotine ion	cf Nicotine salt	cf Nicotine ion	cf Nicotine salt	cf Nicotine ion
	0.107	0.150	1.2	1.7	4.3	6.0
	0.075	0.105	1.5	2.1	2.8	3.9
	0.66	0.92	25	35	100	140
	5.0	7.0	>55	>77	63	88
	0.18	0.25	1.0	1.4	0.73	1.02
	0.37	0.52	4.6	6.4	9.3	13.0
	1.5	2.1	25	35	16	22
	0.063	0.088	1.0	1.4	17	24
	5.0	7.0	55	77	41	57
	0.10	0.14	0.28	0.39	0.06	0.08
	0.38	0.53	4.2	5.9	29	41
	0.30	0.42	8.7	12.2	14	22



is true the pharmacological activities ought to be expressed in terms of the ion and in Tables 17 and 18 the results are set out, for comparison with the activity both as the ion and as the total salt. In fact the activity per ion is not greatly different from the activity per molecule of salt.

Table 19



Methylene Series

Activities of  $\alpha$  and  $\gamma$  compounds relative to  $\beta$  compounds:-

(a) per ion

Side Chain	Rat Diaphragm			Chick Biventer			Cat Ganglion		
	$\alpha$	$\beta$	$\gamma$	$\alpha$	$\beta$	$\gamma$	$\alpha$	$\beta$	$\gamma$
$-N^+ \begin{smallmatrix} \text{CH}_3 \\ \text{CH}_3 \end{smallmatrix}$	0.21	1.0	1.0	0.04	1.0	0.11	0.14	1.0	0.05
$-N$ 	0.2	1.0	0.16	0.05	1.0	0.04	0.05	1.0	0.01
$-N$ 	0.1	1.0	0.39	0.02	1.0	0.08	0.006	1.0	0.02
$-N^+ \begin{smallmatrix} \text{CH}_3 \\ \text{CH}_3 \\ \text{CH}_3 \end{smallmatrix}$	0.04	1.0	2.8	0.02	1.0	1.0	0.01	1.0	0.04

(b) per total salt

Side Chain	Rat Diaphragm			Chick Biventer			Cat Ganglion		
	$\alpha$	$\beta$	$\gamma$	$\alpha$	$\beta$	$\gamma$	$\alpha$	$\beta$	$\gamma$
$-N^+ \begin{smallmatrix} \text{CH}_3 \\ \text{CH}_3 \end{smallmatrix}$	0.23	1.0	0.76	0.04	1.0	0.09	0.15	1.0	0.04
$-N$ 	0.22	1.0	0.12	0.06	1.0	0.03	0.06	1.0	0.01
$-N$ 	0.11	1.0	0.36	0.02	1.0	0.08	0.006	1.0	0.02
$-N^+ \begin{smallmatrix} \text{CH}_3 \\ \text{CH}_3 \\ \text{CH}_3 \end{smallmatrix}$	0.04	1.0	2.8	0.02	1.0	1.0	0.01	1.0	0.04



Activities of  $\alpha$  and  $\gamma$  compounds relative to  $\beta$  compounds:-

(a) per ion

Side Chain	Rat Diaphragm			Chick Biventer			Cat Ganglion		
	$\alpha$	$\beta$	$\gamma$	$\alpha$	$\beta$	$\gamma$	$\alpha$	$\beta$	$\gamma$
$\text{-N}^+\text{(CH}_3)_2$	0.52	1.0	0.39	0.37	1.0	0.03	0.04	1.0	0.01
$\text{-N}^+\text{(C}_6\text{H}_{11})$	1.50	1.0	0.46	1.8	1.0	0.00	0.80	1.0	0.13
$\text{-N}^+\text{(C}_4\text{H}_9)$	1.0	1.0	0.48	0.75	1.0	0.00	0.96	1.0	0.16
$\text{-N}^+\text{(CH}_3)_3$	0.02	1.0	—	0.005	1.0	—	0.001	1.0	—

(b) per total salt

Side Chain	Rat Diaphragm			Chick Biventer			Cat Ganglion		
	$\alpha$	$\beta$	$\gamma$	$\alpha$	$\beta$	$\gamma$	$\alpha$	$\beta$	$\gamma$
$\text{-N}^+\text{(CH}_3)_2$	0.52	1.0	0.38	0.36	1.0	0.03	0.04	1.0	0.01
$\text{-N}^+\text{(C}_6\text{H}_{11})$	1.50	1.0	0.46	1.80	1.0	0.00	0.83	1.0	0.14
$\text{-N}^+\text{(C}_4\text{H}_9)$	1.0	1.0	0.48	0.76	1.0	0.00	0.96	1.0	0.16
$\text{-N}^+\text{(CH}_3)_3$	0.02	1.0	—	0.005	1.0	—	0.001	1.0	—

## II Relationships between Structure and Activity

From Tables 17 and 18 it should be possible to see whether the results support the hypothesis that nicotine-like activity is associated with a partial positive charge at a suitable position in the molecule.

### (a) The Effect of Position of the Side Chain -

If the charge were that located on the 2 or the 4 carbon atoms in the pyridine ring, it would be expected that the  $\beta$  compounds should be more active than the  $\alpha$  or the  $\gamma$  isomers. Tables 19 and 20 show the activity of the  $\alpha$  and the  $\gamma$  compounds expressed in terms of that of the  $\beta$  compound. Without exception in the series of pyridyl methyl dialkylamines the  $\beta$  isomer is the most active. This is shown most clearly in the experiments on the chick biventer and cat ganglion. On the rat diaphragm the differences between the activity of the isomers are not so great.

In the series of pyridyl ethyl dialkylamines activity is maximal at the  $\beta$  isomer of the dimethylamino series but in the two other series examined considerable activity is also found on all preparations with the  $\alpha$  compounds. (This will be commented upon later when considering structural /

structural differences between the methylene and the ethylene derivatives).

(b) The Effect of Chain Length

- (i) Tertiary Compounds. On the rat increasing the chain length from methylene to ethylene does not affect activity except in the  $\alpha$  isomers in which activity is increased. On the chick the  $\alpha$  pyridyl ethyl dialkylamines are more active than the  $\alpha$  pyridyl methyl dialkylamines. With the  $\beta$  and  $\delta$  derivatives the methylene and ethylene linked dimethylamino compounds are similar in activity,  $\beta$  pyridyl ethylpyrrolidine and piperidine are less active than  $\beta$  pyridyl methylpyrrolidine and piperidine, and  $\delta$  pyridyl ethylpyrrolidine and piperidine are inactive. On the cat the  $\alpha$  pyridyl ethyl dialkylamines were similar in activity to the  $\alpha$  pyridyl methyl dialkylamines.  $\beta$  and  $\delta$  pyridyl ethyldimethylamines were similar in activity to the  $\beta$  and /

and  $\gamma$  pyridyl methyl dimethylamines on the cat, but increasing the chain length from methylene to ethylene causes a big decrease in activity in the  $\beta$  pyrrolidino and piperidino compounds and a smaller decrease in activity in the  $\gamma$  pyrrolidino and piperidino compounds.

- (ii) Quaternary Compounds. The  $\alpha$  pyridyl methyl and ethyl trimethylammonium compounds were similar in activity in all three preparations. Increasing the chain length from methylene to ethylene however increased the activity of the  $\beta$  pyridyl trimethylammonium compounds. The  $\gamma$  compounds have not been made.

(c) The Effect of Quaternisation - In the quaternary compounds (such as have been studied) methylation reduces activity in the  $\beta$  pyridyl methyl piperidino compound (especially on the chick and cat) and to a lesser extent in the  $\beta$  pyridyl methyl pyrrolidino analogue (activity in the cat decreased, in the chick unaltered and in the rat increased). Results with nicotine monomethiodide are similar (see Table /

Table 21

## Order of Activity


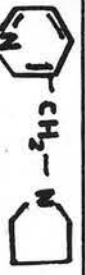
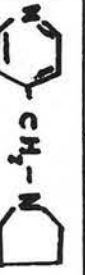
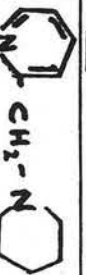
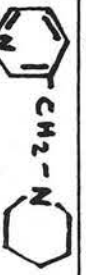
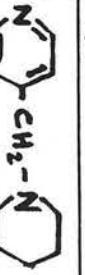
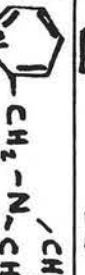
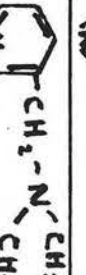
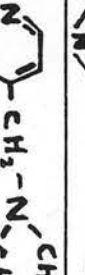
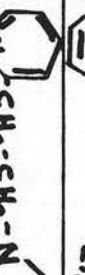
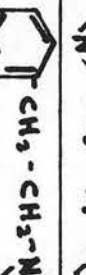
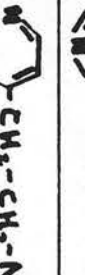
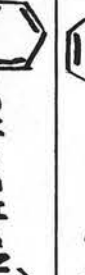
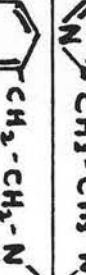
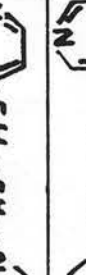
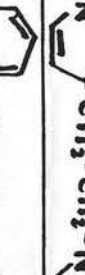
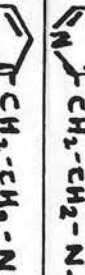

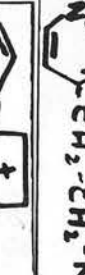
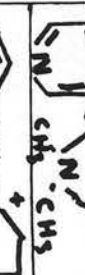
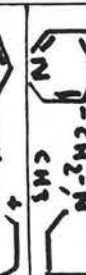
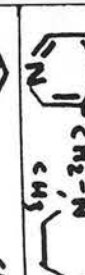








Compound	Rat		Chick		Cat		
	salt	ion	salt	ion	salt	ion	
Nicotine	15	14	2	2	4	3	B
	25	26	25	25	25	25	O
	13	11	7	6	2	2	B
	19	19	12	12	15	14	(b)
	21	21	17	17	16	16	(b)
	10	9	8	7	5	5	(b)
	22	22	23	19	27	27	O
	31	31	28	28	26	24	(b)
	27	26	16	13	12	10	B
	28	25	27	27	31	28	O
	14	15	24	24	20	20	(s)
	15	16	20	21	19	19	O
	20	20	30	30	27	30	S
	9	9	12	14	21	21	S
	11	11	19	19	18	18	S
	17	17	31	31	30	31	S
	29	29	26	26	23	22	O
	26	28	18	18	7	6	B
	30	30	29	29	28	29	O
	4	4	5	5	8	8	(s)
	2	2	6	7	6	6	(s)
	12	13	14	15	24	26	S
	23	23	22	23	22	22	O
	5	5	2	3	3	4	O
	7	7	10	10	9	9	O
	18	18	14	15	11	12	(b)
	1	1	2	3	12	13	S
	23	23	21	22	17	17	(b)
	3	3	1	1	1	1	O
	8	8	9	9	14	15	(s)
	6	6	11	11	10	11	(s)



Table 18) showing an increase in activity compared with nicotine on the rat and a decrease in activity on both the cat and the chick.

In the dimethylamino compounds, however, activity is greatly increased. Rather surprisingly the  $\delta$  isomer ( $\delta$ -pyridyl methyl trimethylammonium bromide hydrobromide) is equiactive with the  $\beta$  isomer on the chick, more active than the  $\beta$  isomer on the rat, but less active on the cat. It is unfortunate that  $\delta$ -pyridyl ethyl trimethylammonium has not been prepared.

(d) Activity Differences in different Tests -

Although the results in the chick and the cat are not dissimilar the results in the rat are often rather different. This is indicated in Table 21 in which the compounds are numbered in order of activity. The activity of some compounds (marked B) is greater on the cat and the chick than on the rat; whereas the activity of others (marked S) is greater on the rat than on the cat.

The compounds marked B are all  $\beta$ -pyridyl derivatives whereas those marked S are a mixed collection containing either  $\delta$  linkages or a piperidyl /



piperidyl group. (These compounds could also have been selected from Tables 17 and 18 where the equipotent molar ratios are given, but it is easier to select them when the numbers in the table are limited to 1 - 31).

The high activity of certain compounds on the diaphragm (particularly  $\chi$  compounds) could be explained by supposing that these acted in a curare-like fashion: with  $\chi$ -pyridyl ethyl pyrrolidine and  $\chi$ -pyridyl ethyl piperidine dihydrobromides this may well be so because these compounds have virtually no effect on the chick biventer. With the quaternary compound  $\chi$ -pyridyl methyl trimethylammonium bromide hydrobromide, however, this seems unlikely as it does cause contracture of the chick muscle. The possible interference with the release of transmitter should also be considered. These points ought to be studied properly. It seems fairly certain at least that anticholinesterase properties do not seriously upset structure activity relationships because the relative activities of  $\alpha$  pyridyl methyl pyrrolidine and piperidine and  $\alpha$  pyridyl ethyl pyrrolidine and piperidine are not greatly different on the three preparations used. It may be, of course, that /

that these would be more active on the rat but for their anticholinesterase effects.

#### Points to be explained

The results, therefore, seem to present the following main problems (i) Why are the

$\gamma$ -pyridyl compounds (especially  $\gamma$  pyridyl methyl trimethylammonium bromide hydrobromide) so active on the rat, (ii) why does lengthening the chain by  $-\text{CH}_2-$  increase the activity in the  $\alpha$ -pyridyl alkyl derivatives of pyrrolidine and piperidine and (iii) why does lengthening the chain increase the activity of the  $\gamma$ -pyridyl derivatives of dimethylamine especially of the quaternary salt? It may be possible to obtain the answers to these questions by considering the structures of the ions concerned.

#### III Spacial Considerations

There is a considerable difference between the shapes of the methylene and ethylene compounds. In the former the basic group is held at an angle to the pyridine ring. In the tertiary cation there is rotation about the C-C and the C-N bonds but this is restricted to some extent especially in the piperidino and pyrrolidino compounds.

The /

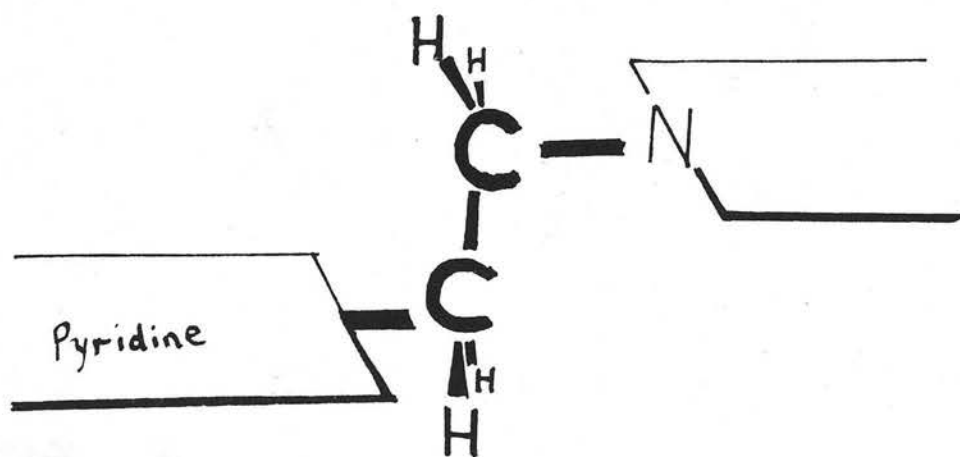


Fig. 5

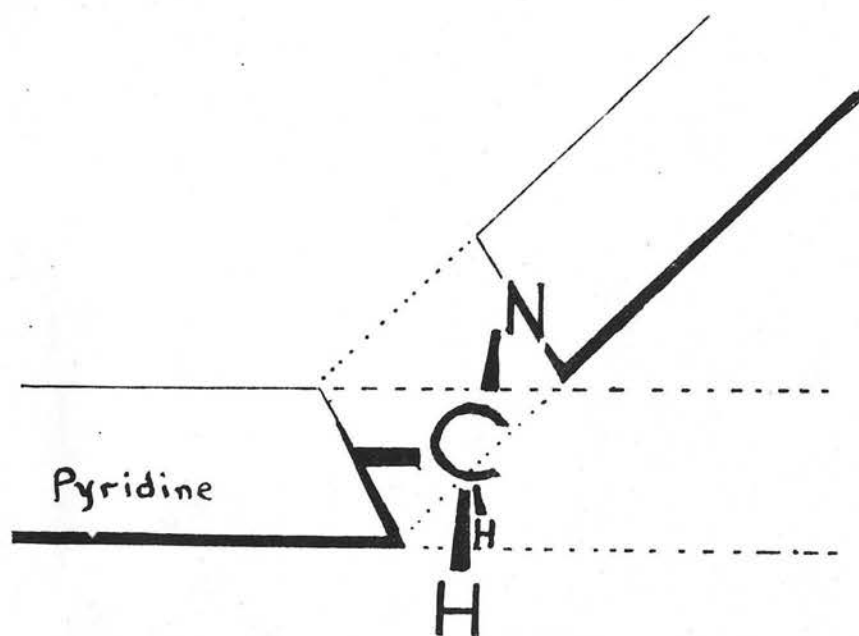


Fig. 4.

The most likely configuration of the piperidine compound is that with the ring inclined at an angle of  $45^{\circ}$  to the plane of the pyridine ring (see Figure 4) whereas the pyrrolidine ring is inclined at  $15^{\circ} 48'$ . The piperidine ring can rotate about the C-N bond until it is approximately at right angles to the pyridine ring but the pyrrolidine ring cannot. This is also true for the quaternary methyl compounds.

The fact that the pyrrolidyl compounds are active even though they are only slightly buckled suggests that it is unlikely that the bent form of the piperidyl compound is important. This idea is confirmed by the activity of the ethylene compounds where the bulk of the basic group can be more or less in the same plane as the pyridine ring (i.e. parallel to it - see Figure 5 ).

Importance of Side Chain Nitrogen Atom - The fact that the activities of the compounds of the pyrrolidyl and piperidyl series are more or less the same even although the arrangements of the groups are different suggests that it is not the position of the bulk of the ring which is important so much as the position of the nitrogen atom.

Effect /

### Effect of Quaternisation of the Side Chain

Nitrogen - It appears, however, that the effect of quaternisation may be a steric one, for it is different in the three series, dimethylamino, pyrrolidino, and piperidino. There seems to be a definite limit to the bulk of the groups which may be attached to the nitrogen without losing activity, even on the rat diaphragm (eg  $\beta$ -pyridyl methyl methylpiperidinium bromide hydrobromide). This receives further support from the decrease in activity of  $\beta$  pyridyl methyl trimethylammonium on successively replacing the onium methyl groups by ethyl groups.

Importance of Pyridine Ring - The pyridine ring is obviously important because the analagous phenyl compounds are definitely less active. The differences between the pyridine ring and the benzene ring are few. The rings are almost identical in size but the electron distribution in the pyridine ring is not uniform: the nitrogen atom carries a partial negative charge and the 2, 4 and 6 carbon atoms have a partial positive charge. From the activity of the choline phenyl ethers it seems unlikely that it is the negative charge on the nitrogen which is important. This leaves the partial positive charges in the 2, 4 and 6 positions to account for the activity of the compounds.

Although /

Although up till now the charges on the 2 and 4 position have been considered important serious consideration must be given to the possibility that it is the charge on the 6 carbon atom which is important. The effect of the position of the side chain on the activity of the pyridylmethyl dialkylamines could be the same whether it is the 6 position or the 2 or 4 position which is important. It would, however, have been expected in these circumstances that the differences between the activities of the  $\alpha$ ,  $\beta$  and  $\gamma$  isomers would not be so great as if it were the 2 or 4 carbon atoms in the ring which were involved. This is actually what is found in the rat diaphragm where, compared to the other two preparations, the activity of the  $\alpha$ ,  $\beta$  and  $\gamma$  isomers is not greatly different. This may also explain the high activity of the  $\gamma$  pyridyl compounds on the rat especially the  $\gamma$ -pyridyl methyl trimethylammonium derivative.

Further in rat diaphragm test the  $\alpha$  ethylene compounds are about as active as the  $\beta$  methylene ones. The distance between the nitrogen and the 6 carbon atom in these compounds is about the same. If, however, in the /



the rat the receptor groups were closer so that the 2 or 4 carbon atoms were important it would be expected that the  $\beta$  methylene compounds would be much more active than the corresponding  $\alpha$  ethylene compounds as they have two positive centres in same relative position whereas in the  $\alpha$  ethylene compounds there is only one positive centre. The  $\beta$  methylene compounds, therefore, could be attached in either of two positions whereas the  $\alpha$  ethylene compounds could only be attached in one.

On the chick and the cat preparations, in fact, the  $\alpha$  ethylene compounds are less active than the corresponding  $\beta$  methylene ones. This combined with the much greater activity of the  $\beta$  pyridyl methyl dialkylamines relative to the  $\alpha$  and  $\delta$  isomers (see Table 19) suggests that here the points of attachment of the receptor surface are much closer than in the rat diaphragm. Consequently attachment in the cat and chick may occur at the side chain nitrogen and the 2 or 4 carbon atoms rather than the 6 carbon atom.

There is no reason to suppose that the distances between the points of attachment on the receptor are exactly those which fit the above drugs. For instance the greater activity of  $\beta$ -pyridyl ethyl trimethylammonium over  $\beta$ -pyridyl methyl trimethylammonium may be because //

because the points of attachment on the receptor are slightly further apart than the distance between the onium group and the 2 or 4 carbon atoms in  $\beta$  pyridyl methyl trimethylammonium; but another possibility may be that the explanation is connected with the greater flexibility of the ethylene compound. In particular the position of the onium group in the methyl compound is fixed fairly rigidly whereas in the ethylene compound the extra link, for example, allows the onium group to assume a position either at right angles to or in the same plane as the pyridine ring.

#### Conclusion:

In conclusion this discussion produces the speculation that in the rat diaphragm the molecule is attached at the cationic head and by a negative centre about  $5^{\circ}\text{\AA}$  away (corresponding to the 6 carbon position of the pyridine ring) whereas in the chick and the cat the receptors are only  $4^{\circ}\text{\AA}$  away (corresponding to the 2 or 4 position of the pyridine ring).

#### IV The Relevance of this Discussion to the activity of related Compounds

The relevance of this discussion to the activity of *m*-hydroxy trimethylammonium (Randall 1950), leptodactyline (Glässer 1960) and choline phenyl ether (Hey 1952) may be considered.

It is unfortunate that no direct comparison has been obtained for the compounds studied here and *m*-hydroxy trimethylammonium and leptodactyline. In these compounds a partial positive charge is located on the ether oxygen atom and the oxygen-onium distance is approximately 5.5<sup>0</sup>A in *m*-hydroxytrimethylammonium and 7<sup>0</sup>A in leptodactyline.

Leptodactyline appears to be particularly active (8 times as active as nicotine on cat nictitating membrane preparation - Erspamer and Glässer 1960). How far this is due to the different size of the partial positive charges relative to  $\beta$  pyridyl ethyl trimethylammonium and how far to the different distance between the partial charge and the onium group it is impossible to say although an indication may be given by a study of analogues of leptodactyline (Erspamer - as yet unpublished).

With /

With choline phenyl ether the oxygen-onium distance is approximately  $4^{\circ}\text{A}$ . On the ganglion this substance was found to be 6 times as active as homocholine phenyl ether in which the oxygen-onium distance is about  $5^{\circ}\text{A}$ . If this speculation is correct the homocholine derivative should be more active on the diaphragm (assuming of course that the compounds are acting in the same way).

One very interesting result obtained in this work is the comparative absence of anticholinesterase activity in the quaternary ammonium derivatives of pyridine - an activity which is marked in the oxygen-containing compounds (eg. *m*-hydroxy phenyl trimethylammonium has a  $K_i$  of  $4.26 \times 10^{-7}$  - Kuperman et al 1961).

Acknowledgment

I should like to thank my supervisors, Professor W. L. M. Perry, and Dr. R. B. Barlow for their assistance and guidance throughout this work.

I am indebted to Professor Perry for his instruction in the use of the cat superior cervical ganglion preparation, and to Dr. Barlow for the preparation of all the analogues of nicotine.

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